HANDBOOK OF PROTOCTISTA

THE STRUCTURE, CULTIVATION, HABITATS AND LIFE HISTORIES OF THE EUKARYOTIC MICROORGANISMS AND THEIR DESCENDANTS EXCLUSIVE OF ANIMALS, PLANTS AND FUNGI

A guide to the algae, ciliates, foraminifera, sporozoa, water molds, slime molds and the other protoctists

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Phylum Apicomplexa

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INTRODUCTION

General Characteristics

The traditional Sporozoa, recently named the Apicomplexa, form a phylum of parasitic protoctists defined by a life cycle that begins from an infective, motile form (or zoite); this zoite possesses an apical and typically structured complex in all species of the phylum that is the basis for the name Apicomplexa.

In general, the life cycle has three stages: 1) a growth stage during which there is infection of a host (or host cell) by the zoite, which enlarges and, in many species, undergoes mitotic reproduction (merogony or endogeny); 2) a sexual stage with production of gametes and fertilization to form zygotes enclosed within oocysts; 3) a sporogenesis stage during which there are successive divisions of the sporoplasm within the oocysts to form sporozoites, which are the new infective forms for the next cycle. In many Apicomplexa, the formation of spores (sporocysts) occurs inside the oocysts, which are structural units with a thick wall. Sporozoites develop inside the sporocysts and thus they are sheltered against the external environment when they leave the host. Meiosis is zygotic; all nutrition is symbiotrophic.

The Apicomplexa is a large phylum, subdivided into three classes: Gregarinia (about 500 species), Coccidia (probably over 1,600 species),* and Hematozoa, which includes the orders Haemosporidia (about 200 species) and Piroplasmida (about 100 species); this adds up to 2,400 well described species. As these parasites occur in most invertebrates and vertebrates, and many have a high host specificity, the real number of existing species must be considerably larger.

*According to D. W. Duszynski; Corliss (1984) has estimated "some 5,000 generally accepted species" for the whole phylum.

Occurrence

All Apicomplexa are endoparasites of either invertebrate or vertebrate animals. They exhibit varying degrees of host specificity. Except for *Eimeria*, which can be grown in tissue culture and is available from scientific investigators, apicomplexans cannot be obtained from culture collections. They must be obtained from their host animals.

Literature

Since the impressive review done on all the "Sporozoa" in *Traité de Zoologie* (1953), edited by P. P. Grassé, many excellent reviews on various groups have been published in books on Eimeriidae (Pellérdy, 1963), haemosporidians (Garnham, 1966), coccidia (Hammond and Long, 1973; Long, 1982), and on piroplasms (Krylov, 1981).

History of Knowledge

The first mention of these organisms was by Antony van Leeuwenhoek (1674–1716) who described oocysts of *Eimeria stiedae* in the liver of rabbits, but he did not name the parasite. The phylum Sporozoa was established by Leuckart in 1879; and included only the classes Gregarinia and Coccidia. (All references cited in this chapter that were published prior to 1953 can be found in Grassé, 1953).

Apparently, the first scientifically recognized species was a gregarine of the genus *Gregarina*, described by Dufour (1828) in an insect digestive tract. But it was von Kolliker (1845–1848) who reported the unicellular character of gregarines and their membership in the Protozoa. The Gregarinae was proposed by Haeckel (1866), later called the Gregarinidae by Lankester (1885).

Establishment of the Coccidia was more confused. Hake (1839) described oocysts of the rabbit coccidian *Eimeria stiedae* without understanding that the species was a parasite. Muller

(1841) placed them in the Protozoa, but confused them with myxosporidian cysts. Linderman (1865) considered these parasites as gregarines and named them *Monocystis stiedae*. Finally, Leuckart (1879) transferred them into the Coccidia, naming them *Coccidium oviforme*. It was in 1907 that the correct name of *E. stiedae* was established, but the taxon *Coccidiomorpha* was proposed by Doflein as early as 1901.

The first species assigned to the Piroplasmida was *Dactylosoma ranarum*, described by Lankester (1871) in red blood cells of a frog. However, this species is, in fact, a coccidian (Boulard *et al.*, 1982) and the first real piroplasm was a cattle parasite described by Babes (1888), this genus was named *Babesia* by Starcovici (1893). This species was the first to be demonstrated as being transmitted by an arthropod vector, a tick (Smith and Kilborne, 1893). Patton (1895) proposed the species name *Piroplasma*, but Wenyon (1926) placed the whole group under this taxon.

Recognition of the Haemosporidia occurred at the end of the 19th century. In 1880 the French physician Laveran showed the parasitological character of malaria afer a careful examination of red blood cells of patients. At the same time the Russian physiologist Danilewsky (1885–1889) discovered other species in the blood of various vertebrates and included them under the name haemosporidians. Transmission of the infectious organisms by mosquitoes, suspected for a long time by native Africans, was suggested by Laveran as early as 1884, but its scientific demonstration was not realized until twelve years later.

After this first period of initial discoveries many new species were described, parasites of various vertebrate and invertebrate animals. Life-cycles, with successive sexual and proliferative generations, were progressively elucidated. Yet the life cycles of some species and groups of medical or veterinary importance (toxoplasms, sarcosporidians, piroplasms) have been worked out only recently. Their ultrastructure and life cycle details permit us to classify these parasites, previously considered as "incertae sedis," within the Apicomplexa.

The systematics of the apicomplexans is continually changing. The main taxonomic publications, with reviews of the major known species, were successively provided by Labbé (1899), Minchin (1903), Wenyon (1926), and Grassé (1953). Discoveries during the past 20 years, aided by electron microscopic studies, have produced many modifications. They have given us a more accurate definition of the Apicomplexa and have helped also in the systematics of the group. For example, *Toxoplasma* and sarcosporidians are now included in the Coccidia, and piroplasms are now thought to be typical Apicomplexa with affinities to Haemosporidia.

During this same period, the homogeneous character of some of the organisms considered to be sporozoa was demonstrated by observations of the similarity in zoite ultrastructure. These observations have revealed a characteristic ultrastructural organization always with the same apical complex (see below); this latter character stimulated Levine (1970) to propose the new name Apicomplexa for the sporozoans.

Practical Importance

The Apicomplexa include causal agents of many important diseases of humans and domestic animals. Although the gregarines apparently do not cause serious damage to their invertebrate hosts, other apicomplexans are often pathogens. Coccidia belonging to the genus *Eimeria* are agents of a disease called coccidiosis, which develops in various vertebrates and causes severe damage in domestic animals (e.g., rabbits, chicken, cattle). Now classified with coccidians are the agents of toxoplasmosis (*Toxoplasma gondii*), a human disease, and of sarcosporidiosis (*Sarcocystis*), which contaminates various mammals including some domestic animals (e.g., sheep, pigs). *Cryptosporidium* spp. produces diarhhea which has particularly acute choleralike symptoms in people with autoimmune deficiency (DuPont, 1985). The haemosporidia include *Plasmodium*, which is the agent of malaria. Piroplasms are responsible for piroplasmosis infections (babesiosis and theleriosis), an illness essentially of domestic animals (cattle, dogs), but sometimes of humans.

HABITATS AND ECOLOGY

All Apicomplexa are endoparasites of invertebrate or vertebrate animals. It has been thought that members of any group of animals, except two phyla of acoelomates, cnidarians (coelenterates), and ctenophorans, may be parasitized and that any organ may be affected by apicomplexans. However, a scleractinian coral (a member of the class Anthozoa in the phylum Cnidaria) has recently been found to be parasitized by coccidians (Upton and Peters, 1986).

Distribution of Apicomplexa in the Kingdom Animalia

Gregarines are found exclusively in invertebrates and prochordates. The most affected groups are worms and arthropods.

In general, a rather close specificity exists between the parasite and the host; each gregarine species is often linked to one host species. Nevertheless, some exceptions to this rule exist. *Gregarina garnhami* infects two species of locust, *Locusta migratoria* and *Schistocerca gregaria*. Moreover, some gregarines are linked with definite groups of invertebrates. For instance, the archigregarines are only known in polychaetes. Although the wide majority of gregarines are monoxenous (the entire life cycle takes place in one host), in some species the life cycle is unknown and may be heteroxenous (the life cycle requires more than a single host).

Coccidia occur throughout the animal kingdom. Many species complete their life cycle in a single host and sometimes have very strict specificity: for instance, the coccidia *Coelotropha durchoni* is a parasite only of the polychaete *Nereis diversicolor*, and *Eimeria necatrix* is a parasite of the chicken *Gallus domesticus*.

Other species, although monoxenous, are able to develop in several related host species. For example, *Klossia helicina* is found in various species of gastropods belonging to the Helicidae; similarly, *Eimeria stiedae* occurs in domestic rabbits as well as in hares and American cottontail rabbits (*Sylvilagus*).

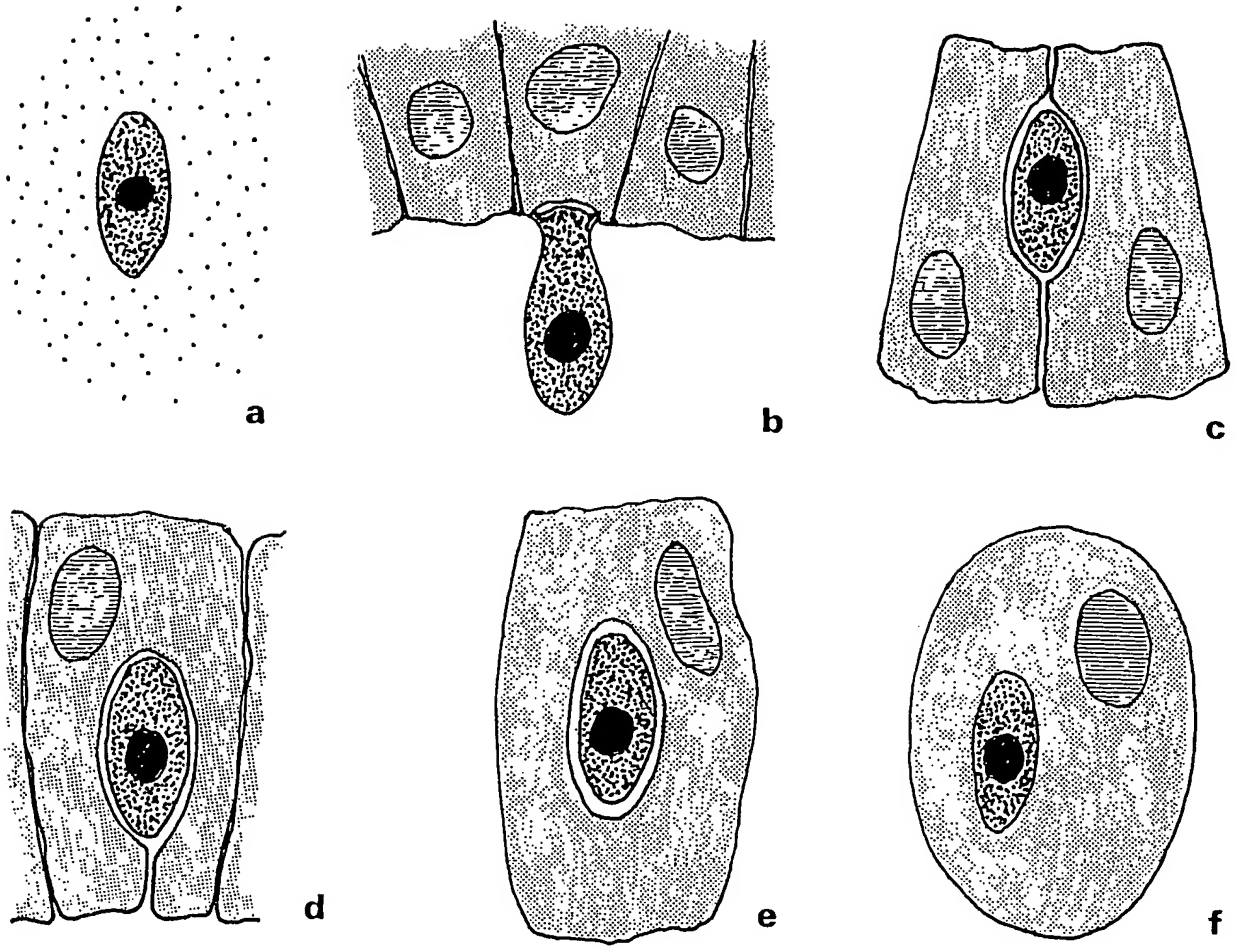


Fig. 1. Localization of the parasite: a. extracellular and free; b. extracellular and fixed on epithelial cell; c. extracellular and intratissular; d. extracellular pseudo-intracellular; e. intracellular in cytoplasmic vacuole (parasitophorous vacuole); f. intracellular without vacuole.

Many heteroxenous species are known in invertebrate as well as vertebrate hosts. *Aggregata eberthi* develops its growth stage and undergoes schizogony in various crabs of the genus *Portunus*, whereas its sexual stage occurs exclusively in the cuttlefish. The best known examples of coccidia in vertebrate hosts are dozens of *Eimeria* species from chickens, goats, sheep, rats, and other domestic mammals and birds. Also well known are the genera *Toxoplasma* and *Sarcocystis*, whose life cycles have only recently been discovered. *Toxoplasma gondii* undergoes the sexual portion of its cycle in the cat but an asexual phase may occur in various mammals (e.g., humans, mouse) and even in birds. Pigs and people are hosts for *Sarcocystis mescheriana*, and sheep, dogs and cats are hosts for *S. tenella*.

Hematozoa are all heteroxenous, with part of their life cycle (merogony) in a vertebrate host and part (sexual phase and sporogenic phase) in an arthropod vector (mosquitoes or ticks).

Here also, zoological specificity varies according to the species. For example, *Plasmodium falciparum* is exclusively a parasite of humans in contrast to *P. berghei*, which may infect many small wild mammals belonging to various rodent families and even bats.

The zoological specificity of piroplasms also seems to vary with species. *Babesia bigemina* may infest ox, zebu, water buffalo, and deer (*Massama americana reperticia*), while other species have been described in only one host. We emphasize the difficulty in identifying species of these intraerythrocytic parasites because of their very small size.

Localization in the Host

All organs except the skeleton may be infected by apicomplexans. Zoites or infectious forms always have a free motile stage that searches for the target organ in which development will be completed. Development, which may be extracellular or intracellular, may be completed in one or in several places (Fig. 1), depending on the stage in the life cycle.

Extracellular Parasites. Parasites developing in the lumen of various organs or cavities are always extracellular (Figs. 1a,b). Most of the gregarines and some coccidians are found in the digestive tract. They are also found in the urinary passage, the ducts of excretory organs, in the respiratory tracts, and in the coelom of their hosts. Parasites of the lumen may be unattached or attached to epithelial cells. The position of the parasite inside the cavity varies according to species. In general, modifications

occur in the contact area between the parasite and the host cell; a tight junction between the host cell membrane and the parasite membrane forms in the area of the contact zone.

Tissue and Intracellular Parasites. Some gregarines, most coccidians and all hematozoans are located inside host cells. Intracellular parasites may be found either inside a parasitophorous vacuole (vacuole belonging to the host cell and containing the parasite), for example in intracellular gregarines, coccidians, and hemosporidians (Fig. 1e); or, as are piroplasms, the apicomplexan may be in direct contact with the cytoplasm of the host cell (Fig. 1f). The presence of a membrane around many parasites may cause confusion between a real intracellular position and a pseudo-intracellular (Fig. 1d) or tissue location (Fig. 1c). Some gregarines described as intracellular are probably located intercellularly in tissue.

Although epithelial cells of internal organs are the most frequently infected by apicomplexans such as coccidians of the genus *Eimeria,* all the organs may be affected: cells of liver, kidney, and excretory systems, brain, genital apparatus and gametes, and muscles. Haemosporidians and piroplasms tend to be found most frequently in blood cells (erythrocytes and leucocytes).

"Latent Forms." The latent forms develop slowly and may persist for some time without growing. These forms may occur at various stages and host locations in the life cycle of the parasite, according to species. The main latent form in most gregarines and coccidians is the sporocyst, which is passed to the external environment and awaits ingestion by a new host. This sporocyst may be the oocyst itself as it is in gregarines, or it may be part of a divided oocyst, covered by a protective shell, as in coccidians.

Latent forms may exist at other stages in the life cycle: at the beginning of the infection as well as during the growth phase. The sporozoite of the coccidian *Coelotropha durchoni,* which is liberated in the digestive tract of a worm, enters a coelomic cell and waits for about 2 years, failing to develop until the sexual maturity of its host. In *Toxoplasma gondii* latent cysts occur after endogenous multiplication of zoites (see *The Life Cycle,* p. 553) and settle mainly in the brain, remaining in a state of slowed development. They may later cause a new infection of the host, or renew their activity after their host is ingested by a predator.

Geographical Distribution and Trophic Factors. The geographical distribution and, for heteroxenous parasites, the extension of a parasitized area depends on the extent of overlapping of the ranges of each host.

Parasite transmission always depends on trophic relations. With monoxenous species parasite transmission occurs by ingestion of infectious forms, generally sporocysts, during feeding. The parasitemia level, that is, the number of parasitized animals in a population, is proportional to the density of the host population. This is the case with the coccidian parasite *Coeltropha durchoni* that infests the polychaete *Nereis diversicolor,* which can be particularly abundant and concentrated in estuarine muds.

The most important factor in controlling distribution of heteroxenous species is the predator-prey relationship. Cuttlefish are infected by the coccidian *Aggregata eberthi* by eating parasitized crabs, which themselves have become contaminated by spores from cuttlefish excrement, or by eating dead cuttlefish. Sarcosporidians of humans, pigs, sheep, and dogs show similar relationships. In haemosporidians and piroplasms the mosquito or tick predator becomes contaminated by sucking blood and later inoculates infectious forms into a host during another blood meal. In these last two groups, the trophic linkage exists, but only in one direction: the prey can be infected only by an arthropod bite.

Some species of apicocomplexans have been described only once and seem to be infrequent or to have a very restricted distribution; other ones are widely distributed. The most widespread species seem to be those that affect humans and domestic animals, as man has carried parasites everywhere.

The most widely distributed apicomplexan is probably *Toxoplasma,* which infests a large proportion of people in all countries of the world and which may persist in various animals, almost indefinitely, by asexual multiplication.

After *Toxoplasma,* the most widely distributed apicomplexans are *Plasmodium,* piroplasms (*Babesia* and *Theleria*) and *Eimeria.* The distributions of parasites causing human and domestic animal diseases are rather well-known; on the other hand, the relative abundance is difficult to document for the far less well known parasites specific to wild animals.

Other intrinsic or extrinsic conditions may be required independent of the host presence for the development of the parasite. For example, the gregarine *Diplauxis hatti* develops in its host, the polychaete *Perinereis cultrifera,* only in the absence of the cerebral sexuality-inhibiting hormone of the worm; *Plasmodium falciparum* needs a higher temperature than *Plasmodium vivax* for its development, which explains the more restricted geographical distribution of the first (in the warmest countries) compared with the second species.

CHARACTERIZATION AND RECOGNITION

The Apicomplexa are characterized by the peculiar organization of the developmental stages of their life cycle and most especially by the structure of their infective stages, the zoites, which are very similar in all members of the phylum.

The Zoite

The zoites are elongated cells averaging 1.5 to about 20 μm in length according to the species. Their anterior part contains peculiar organelles constituting the "apical complex" (Fig. 3). The occurrence of the complex is considered as most significant as regards the definition and identification of Apicomplexa. It consists of 1) a polar ring connected with microtubules extending backward under the cell surface; 2) two apical (= conoidal) rings and a cone-shaped structure, the conoid, made up of several spirally arranged microtubules; and 3) pedunculate organelles (usually two) called rhoptries.

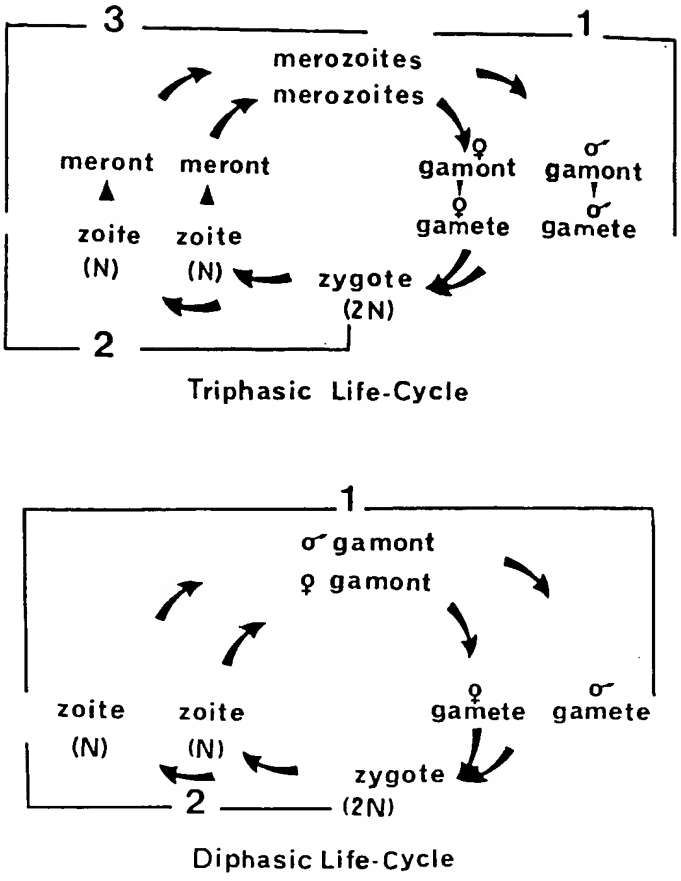


Fig. 2. The apicomplexan life cycles. 1. gamogony; 2. merogony; 3. sporogony.

The polar ring is interpreted as a MTOC (microtubule organizing center) by Russell and Burns (1984). The conoid and conoidal rings occur in gregarines and coccidians but are lacking in the hematozoans (haemosporidians and piroplasms). The rhoptries are considered to be secretory organelles releasing lytic products during host cell invasion, as reported in the coccidian *Toxoplasma gondii* (Nichols *et al.*, 1983).

The zoites also contain dense bodies, the micronemes, which are more abundant in the apical complex area. They probably correspond to golgi secretions. Specific micronemal proteins were detected by electrophoretic analyses but their function has not yet been elucidated. Mitochondria, endoplasmic reticulum cisternae, and a golgi apparatus anterior to the nucleus are commonly reported. The zoites are bounded by a pellicle consisting of the cell membrane and two closely associated membranes which form the "inner membrane complex." The pellicle is interrupted by one or more micropores (for details see Scholtyseck in Hammond and Long, 1973).

The zoites are produced by two processes occurring during the life cycle: sporogony and merogony. Those arising from sporogony are called sporozoites whereas the others are merozoites. All of them exhibit similar features (Figs. 4, 5).

The Life Cycle

The life cycle consists of two or three successive phases (Fig. 2): the sexual phase, also called gamogony; the sporogenic phase, or sporogony; and in some Apicomplexa, the growth phase or merogony.

The Growth Phase. The growth phase corresponds to the development of the parasite after entry into the host. It usually begins with the transformation of the sporozoite into a larger organism called a trophont. Trophonts are characterized by the resorption of apical complex organelles in most cases, the loss of motility, the enlargement of the nucleus and the nucleolus, and the occurrence of numerous micropores. Metabolic precursors such as amino acids are ingested from the host through the latter. In gregarines and coccidians, the metabolism of trophonts results in the storage of polysaccharides represented by amylopectin granules. The hematozoans are unable to accumulate polysaccharides and the sucrose is instead constantly supplied by the host cell (erythrocyte). Their micropores develop into a large cytostome. Thus they engulf the host cytoplasm that contains hemoglobin, phospholipids, sucrose,

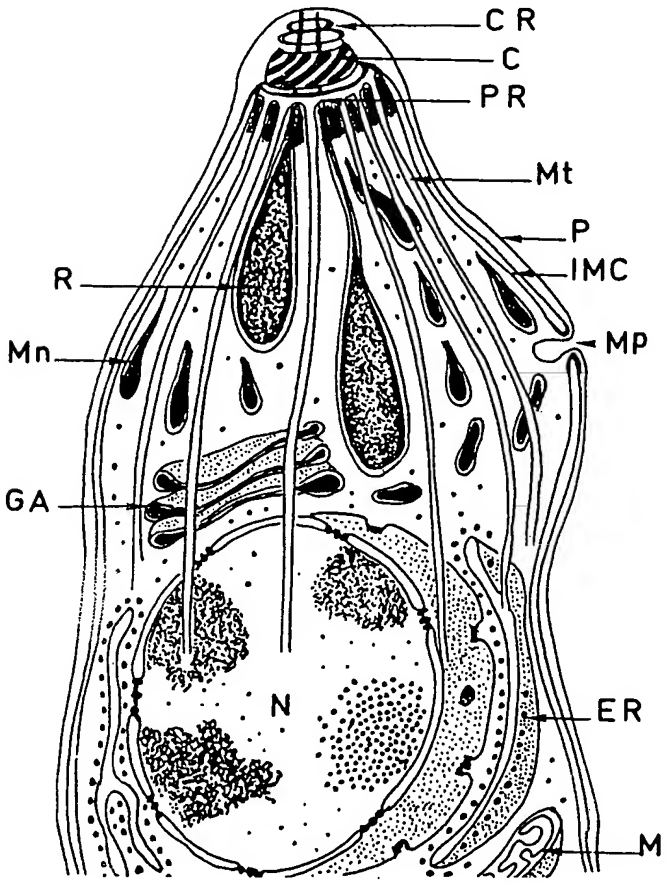
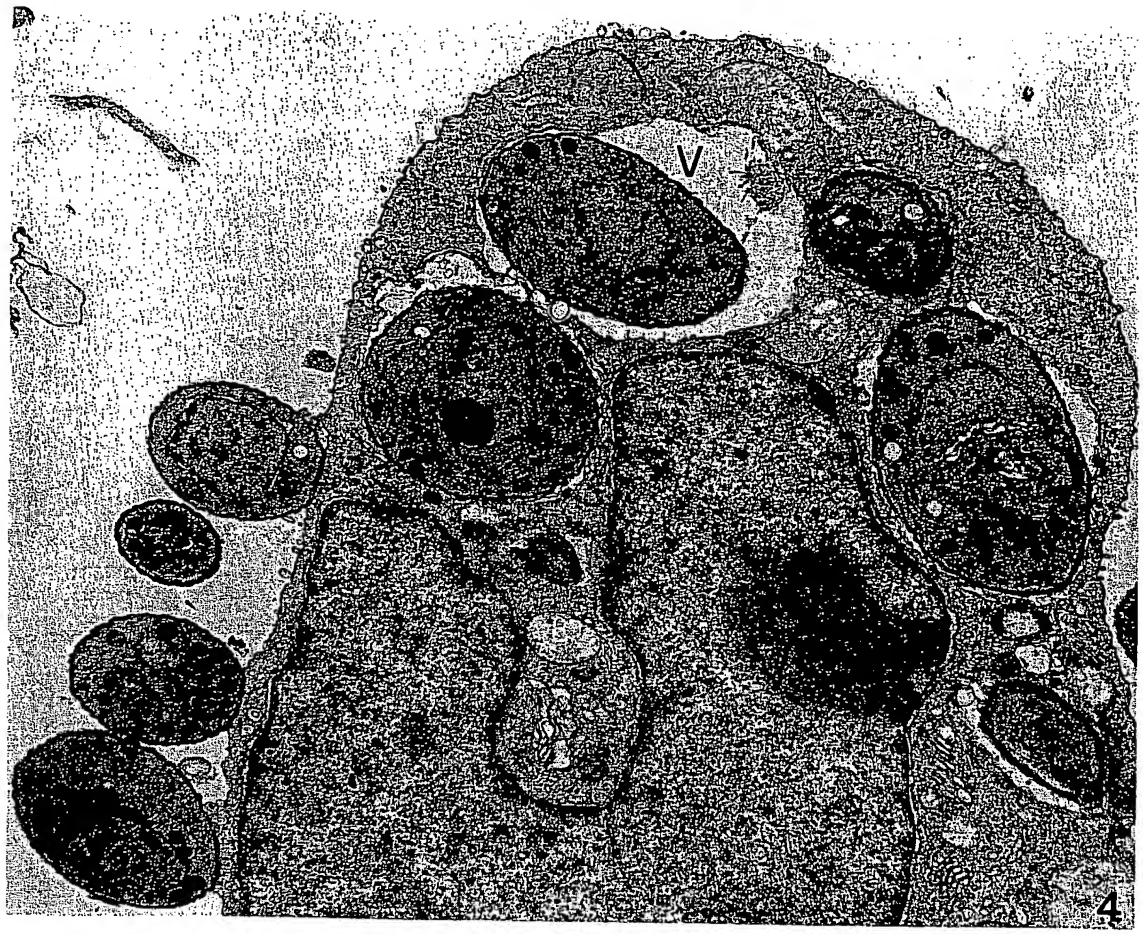


Fig. 3. The anterior part of the zoite. CR, conoidal rings; C, conoid; PR, polar rings; Mt, microtubules; P, plasmalemma; IMC, inner membrane complex of the pellicle; Mp, micropore; R, rhoptrie; Mn, microneme; GA, golgi apparatus; N, nucleus; ER, endoplasmic reticulum; M, mitochondrion.



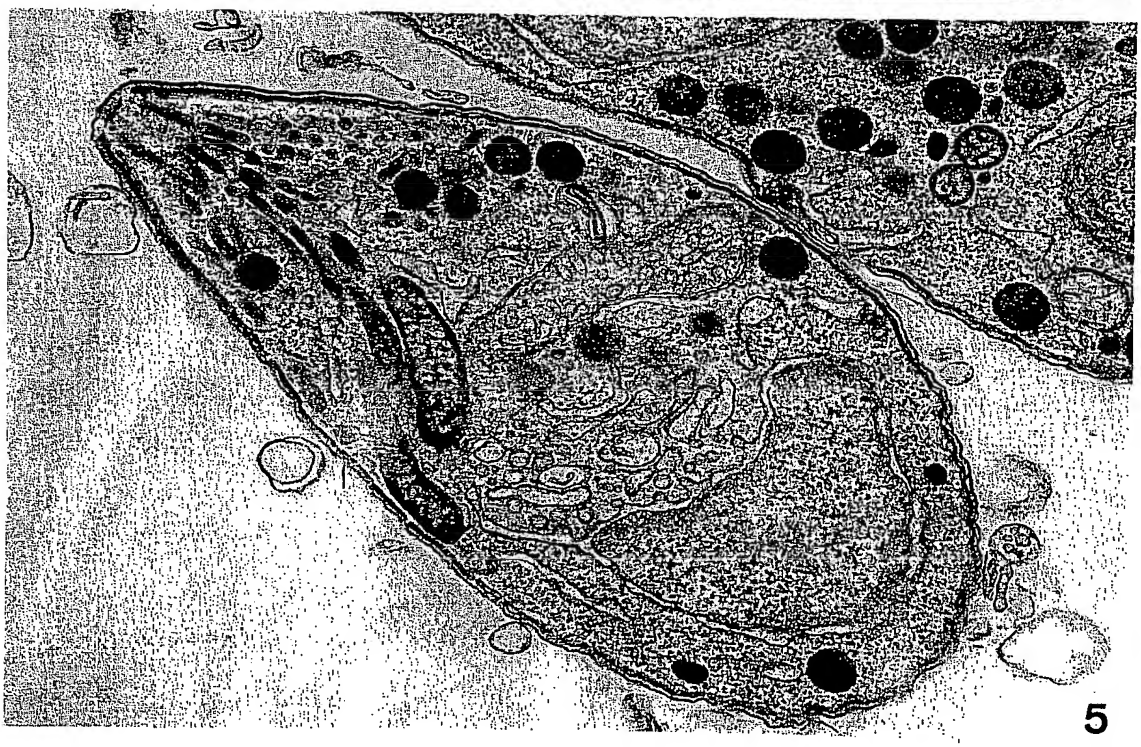


Fig. 4. Invasion of a mouse cell by the zoites of *Toxoplasma gondii*. The intracellular zoites are located in a parasitophorous vacuole (V) (from Vivier, unpublished electron micrograph) × 7,800.

Fig. 5. The zoite of *Toxoplasma gondii* exhibiting typical organelles of the apical complex: anterior conoid, micronemes, spongelike rhoptries, pellicular membranes. Dense rounded bodies, golgi vesicles, a mitochondrion, and the nucleus may also be seen (from Vivier, unpublished) × 26,000.

and amino acids, the latter being also supplied by the digestion of hemoglobin (Seed and Manwell in Kreier, 1977).

Other features of the trophonts and their further development depends upon their extracellular or intracellular location.

Extracellular development:

In most gregarines and in some coccidians such as Coelotrophiida the growth phase proceeds as follows: the sporocysts are ingested and the host digestive processes erode the sporocyst wall. The sporozoites, released from the sporocysts, are motile in the gut. They make contact with epithelial cells. The apical portion in many but not all of these organisms develops into an attachment apparatus, which may be more or less important according to the species (Fig. 12). In the Ganymedidae (Fig. 21) and Lecudinidae, two gregarine families, the attachment apparatus designated by the term mucron corresponds to the flattened anterior portion of the apicomplexan cell and its adherence to a gut epithelial cell. In other gregarines, the attachment apparatus develops into an anchoring organelle, the epimerite, which plunges into the epithelial cell and exhibits various and specific shapes (Fig. 21). The apical complex usually disappears during the formation of the attachment apparatus. Thus attached to the gut wall, the sporozoites develop into large, extracellular trophonts that accumulate amylopectin granules in their cytoplasm while the envelope of their large nucleus is reinforced with an inner fibrillar layer. The gregarine trophonts are characterized by pellicular folds (Fig. 13). The occurrence of these longitudinal folds may be associated with the motility of the trophonts, which move freely in the lumen of the gut when they detach from the degenerate epithelial cells.

In gregarines such as the Cephaloidophoridae, parasites of Crustacea, and the Gregarinidae, harbored by most insects (Fig. 21), the trophonts are associated in chains of usually two individuals during their growth. In others such as Lecudinidae, Stylocephalidae, or Actinocephalidae (see Table 1), the trophonts develop solitarily.

Extracellular apicomplexans such as some gregarines, i.e., the Diplocystidae, parasites of insects, and the Monocystidae of terrestrial worms, and coccidians belonging to the order Coelotrophiida, do not develop exclusively in the gut. In many, the sporozoites penetrate the gut wall and develop in the coelom.

The extracellular trophonts exhibit various shapes. Those that develop in the coelom and are nonmotile are frequently spherical. Gregarine extracellular trophonts are rather elongate; they exhibit the appearance of ribbons in the Ganymedidae (Fig. 21). The length of trophonts ranges from 100 μm to 1 or 2 mm, according to the species, and is about 10 mm in *Porospora gigantea*, parasitic in lobsters.

Intracellular development:

The sporozoites of some gregarines (order Neogregarinida), of most coccidians, and of all hematozoans penetrate the host cell and develop into trophonts. The trophonts undergo nuclear divisions (as shown in Fig. 6), thus transforming into a multinucleate stage called a meront (or schizont). The merogenic mitoses are characterized by a peculiar structure called a centrocone. It consists of an extranuclear cone-shaped microtubular spindle adjacent to the nuclear envelope. Centrioles, one in Gregarinia, two in Coccidia, are present at the top of the centrocones. These centrioles are made up of nine singlet tubules instead of the classical triplets. They are not differentiated in Hematozoa.

The nuclear division begins with the duplication of the centrocone (Fig. 6). The growth of the nuclear envelope and the concomitant separation of the overlaying offspring centrocones result in the formation of the two mitotic poles. Microtubules issued from each centrocone penetrate into the nucleus and some of them attach to the kinetochores of the offspring chromosomes. The latter move to the poles correlated with the shortening of these microtubules.

In hematozoans differentiating small or very small centrocones, the nuclear envelope is slightly disrupted in the centrocone region owing to the penetration of microtubules. In Gregarinia, the development of the centrocone microtubules results in the breakdown of the nuclear envelope into fragments that are deposited on the condensed chromosomes. At telophase, these fragments fuse end-to-end, thus re-forming the offspring nuclei envelope (Fig. 6).

In some coccidians and hematozoans the successive divisions of centrocones and chromosomes do not involve the immediate division of the nucleus. The subsequent multipolar nucleus will later undergo multiple division giving rise simultaneously to several offspring nuclei as reported in *Sarcocystis* spp. (Fig. 7).

The nuclear products of karyokinesis lie under the cell membrane in such a manner that subsequent cytokinesis produces a layer of cells budding at the surface of the meront. These cells, which exhibit the features of zoites, are therefore called merozoites (Fig. 9). Their number varies according to the size of the meront. Up to 120,000 merozoites are produced by the meronts of the coccidian *Eimeria bovis* (Hammond, in Hammond and Long, 1973). In the small, binucleate zoitelike meront of *Toxoplasma gondii* only two merozoites are produced by endodyogeny (Fig. 8).

The larger meronts are probably those of the coccidian Aggregatidae (Fig. 10). They begin to develop in the gut epithelium of crustaceans and, after the destruction of the host cell, continue to grow under the basement membrane. Such meronts have the appearance of white masses, protruding into the body cavity of the host.

The production of zoites during the trophic phase is best known as merogony. The merozoites invade neighboring cells and may give rise to a new generation of meronts and merozoites. The repetition of the merogonic processes may result in the destruction of a large number of cells in the host, thus explaining the pathogenicity of many intracellular apicomplexans.

Location of the merogonic stages: In coccidia such as the Eimeriidae, merogony occurs in the intestinal cells of vertebrates, thus causing severe diseases (Ruff and Reid, Todd and Ernst in Kreier, 1977). In others, such as the heteroxenous Sarcocystidae, merogony begins in the intestine of mammals and results in the production of several generations of merozoites that invade other tissues of the host. Finally, the meronts

TABLE 1. Major families of Eugregarinida

Families	Animal hosts*	Localization	Attachment apparatus	Trophonts	Sporulation
1. Lecudinidae Kamm, 1922	An: Polychaeta Echiuria Sipunculida Ch: Tunicata	intestine	mucron	solitary trophonts lacking septum	ellipsoidal sporocysts released by rupture of cyst wall
2. Ganymedidae Huxley, 1910	Ar: Crustacea	intestine	mucron	ribbonlike trophonts lacking septum, associated in pairs early in development	unknown
3. Uradiophoridae Grassé, 1953	Ar: Crustacea	intestine	epimerite	occurrence of a septum in trophonts, associated in pairs	sporocysts with raylike processes, released by rupture of cyst wall
4. Cephaloidophoridae Kamm, 1922	Ar: Crustacea	intestine	epimerite	trophonts with septum, associated in pairs	sporocysts with an equatorial thickening, released in chains by rupture of cyst wall
5. Cephalolobidae Théodoridès & Desportes, 1975	Ar: Crustacea	stomach	anterior part of trophont transformed into a sucker-like structure	septum, associated in pairs	chains of spheroidal sporocysts released by rupture of cyst wall
6. Porosporidae Labbé, 1899	Ar: Crustacea	intestine	epimerite	septum, association of 2 or more trophonts	production of naked zoites in the cyst; possible maturation of released zoites in Mollusca
7. Thalicolidae Théodoridès & Desportes, 1975	Ch: Tunicata	intestine	unknown	septum, association in pairs	unknown
8. Urosporidae Léger, 1892	An: Polychaeta Echinodermata Mollusca	body cavity or intestine	mucron more or less developed	no septum, solitary development	sporocysts with an anterior neck, occurrence of a tail, released by rupture of cyst wall
9. Monocystidae Bütschli, 1882	An: Oligochaeta (terrestrial worms)	body cavity seminal vesicles	mucron	no septum, solitary development	ellipsoidal sporocysts released by rupture of cyst wall
10. Stenophoridae Crawley, 1903	Ar: Diplopoda (millipedes)	intestine	epimerite	septum, solitary development	ellipsoidal sporocysts released by rupture of cyst wall
11. Monoductidae Ray & Chakravarty, 1933	Ar: Diplopoda	intestine	epimerite	septum, solitary development	chains of ellipsoidal sporocysts released by a sporoduct issued from cyst wall
12. Dactylophoridae Léger, 1892	Ar: Chilopoda (centipedes)	intestine	rhizoids	septum, solitary development	cylindrical sporocysts released by rupture of cyst wall

TABLE 1. Cont'd.

Families	Animal hosts*	Localization	Attachment apparatus	Trophonts	Sporulation
13. Gregarinidae Labbé, 1899	Ar: All Insecta	intestine	epimerite	septum, development in pairs	chains of cylindrical or ellipsoidal sporocysts released by several sporoducts
14. Hirmocystidae Grassé, 1953	Ar: All Insecta	intestine	epimerite	septum development in pairs	sporocysts released by rupture of cyst wall
15. Enterocystidae Codreanu, 1942	Ar: Ephemeoptera larvae	intestine	no fixative apparatus	small intracellular trophonts lacking septum, associated in pairs when extracellular early in development	ellipsoidal sporocysts released by rupture of the cyst wall
16. Stylocephalidae Ellis, 1912	Ar: Coleoptera (Tenebrionidae)	intestine	epimerite	septum, solitary development	chains of ellipsoidal sporocysts released by rupture of cyst wall
17. Actinocephalidae Léger, 1892	Ar: Arachnida Insecta	intestine	epimerite	septum, solitary development	sporocysts biconical, spheroidal or ornamented with spines, released by rupture of cyst wall
18. Diplocystidae Bhatia, 1930	Ar: Insecta	body cavity	no fixative apparatus	lacking septum, early association in pairs	ellipsoidal sporocysts released by rupture of cyst wall

*Phyla and selected classes or lower taxa. Abbreviations: An, Annelida; Ar, Arthropoda; Ch, Chordata.

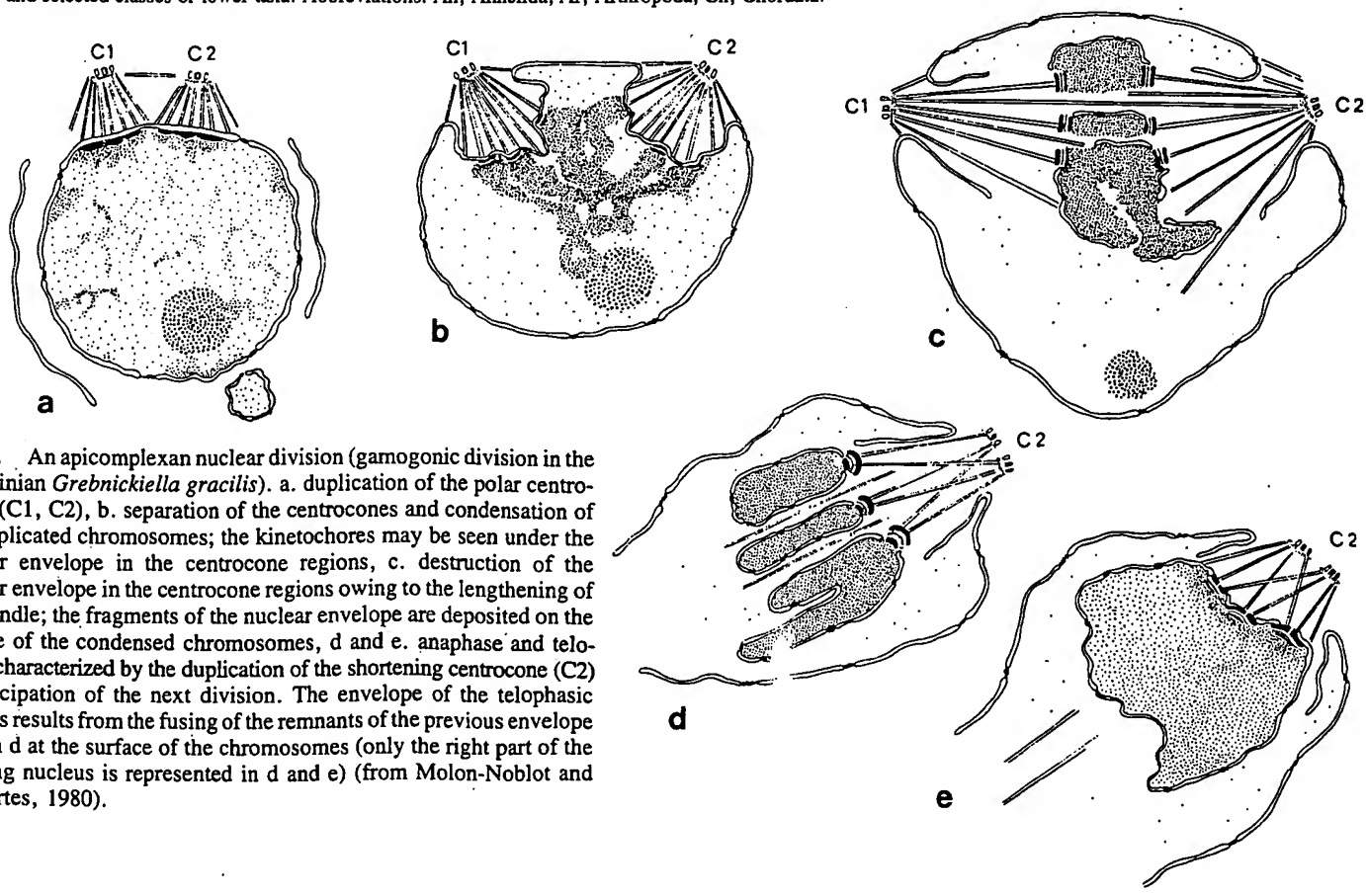


Fig. 6. An apicomplexan nuclear division (gamogonic division in the gregarinian *Grebnickiella gracilis*). a. duplication of the polar centrocones (C1, C2), b. separation of the centrocones and condensation of the duplicated chromosomes; the kinetochores may be seen under the nuclear envelope in the centrocone regions, c. destruction of the nuclear envelope in the centrocone regions owing to the lengthening of the spindle; the fragments of the nuclear envelope are deposited on the surface of the condensed chromosomes, d and e. anaphase and telophase characterized by the duplication of the shortening centrocone (C2) in anticipation of the next division. The envelope of the telophasic nucleus results from the fusing of the remnants of the previous envelope seen in d at the surface of the chromosomes (only the right part of the dividing nucleus is represented in d and e) (from Molon-Noblot and Desportes, 1980).

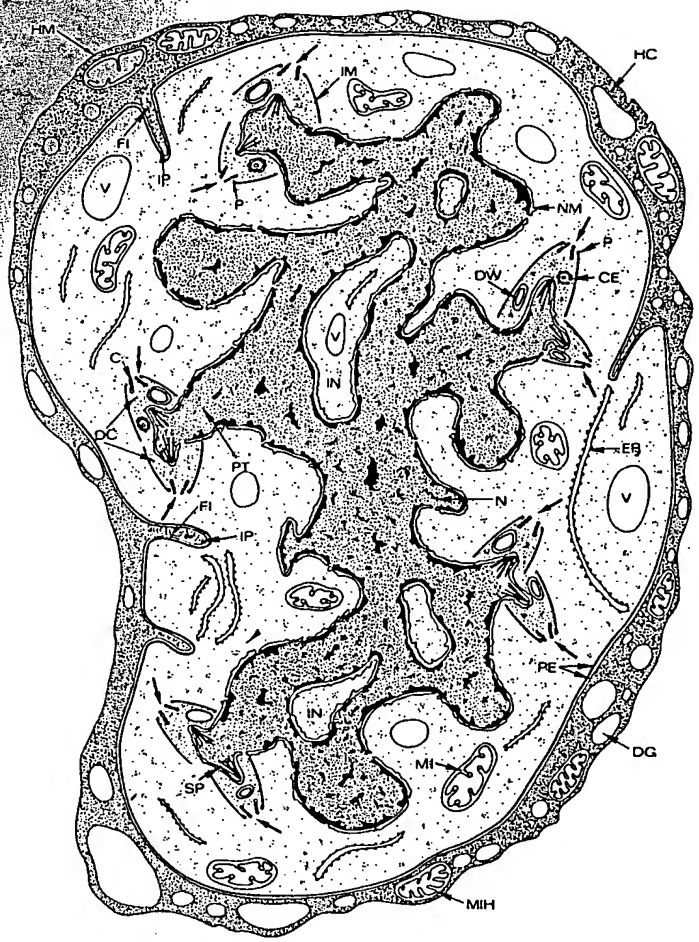


Fig. 7. The multipolar nucleus of the meront in the coccidian *Sarcocystis suihominis*. The merozoites begin to differentiate (arrows) before the complete division of the parent nucleus. C, conoid; CE, centriole; DC, offspring cell (= merozoite); ER, endoplasmic reticulum; HC, host cell; IM, inner membranes of the pellicle; IN, invagination of the nucleus; MI, mitochondrion; N, nucleus; NM, nuclear envelope; PT, protrusion of the nucleus; SP, spindle apparatus; V, vacuole; HM, host membrane; MIH, host mitochondrion (from Heydorn and Mehlhorn, 1978).

encyst within the muscles and give rise to a last generation of zoites (Fig. 22). The sexual stages occur in carnivorous mammals contaminated by the ingestion of muscles containing cysts with zoites (for more details and precise terminology see Dubey in Kreier, 1977).

The merogony of the hematozoans usually occurs in the blood cells of a vertebrate (Fig. 11). The latter are infested by the zoites inoculated by a blood-sucking invertebrate. In members of the genus *Plasmodium*, the first generations of merozoites are usually produced in liver parenchymal cells (exoerythrocytic phase) and the following generations occur in erythrocytes (erythrocytic phase) (see reviews by Ayala, Seed and Manwell, Carter and Diggs, Collins and Aikawa, Rieckmann and Silverman in Kreier, 1977).

The Sexual Phase. The sexual phase or gamogony is initiated by the development of the trophic stages into gamonts. The latter produce gametes which may be highly differentiated.

Two gamogonic processes are distinguished. One, typical of gregarines, consists of the production of an equal quantity of gametes by gamonts of each sex. The other is characteristic of coccidians and hematozoans (except for *Babesia*). It involves the development of the female gamont into a single macrogamete whereas several to hundreds of microgametes differentiate from the male gamont.

a. Gamogony in Gregarinia:

The production of gametes is initiated in all gregarianes, except for the order Blastogregarinida, by the encystment of paired trophonts which are then called gamonts. Many gregarines, such as the Cephaloidophoridae or the Gregarinidae, undergo early pairing, the trophonts being associated during the growth phase (Fig. 21). When the trophonts develop solitarily, as reported in the Stylocephalidae and Actinocephalidae, the pairing occurs when they are fully developed and is immediately followed by encystment (Fig. 16).

Encystment involves the cessation of feeding, the loss of motility, the dedifferentiation of the inner membrane complex of the pellicle, the secretion of a cyst wall, and the alteration of the nucleus. The thick nuclear envelope disappears and the nuclear contents are scattered in the cytoplasm. The chromosomes condense and undergo the series of nuclear divisions resulting in the final production of the gamete nuclei.

The gametes are differentiated according to a process similar to that previously reported in the merogony. The gamonts become multinucleate after the series of nuclear divisions. The gametes originate from the offspring nuclei situated just beneath the membrane bounding the cytoplasm of the gamonts. These nuclei are incorporated in outgrowths that protrude at the surface of the gamonts. Then the nucleated outgrowths detach from the residual cytoplasm, thus forming small cells which are the gametes (Fig. 17). The latter accumulate in the cyst cavity.

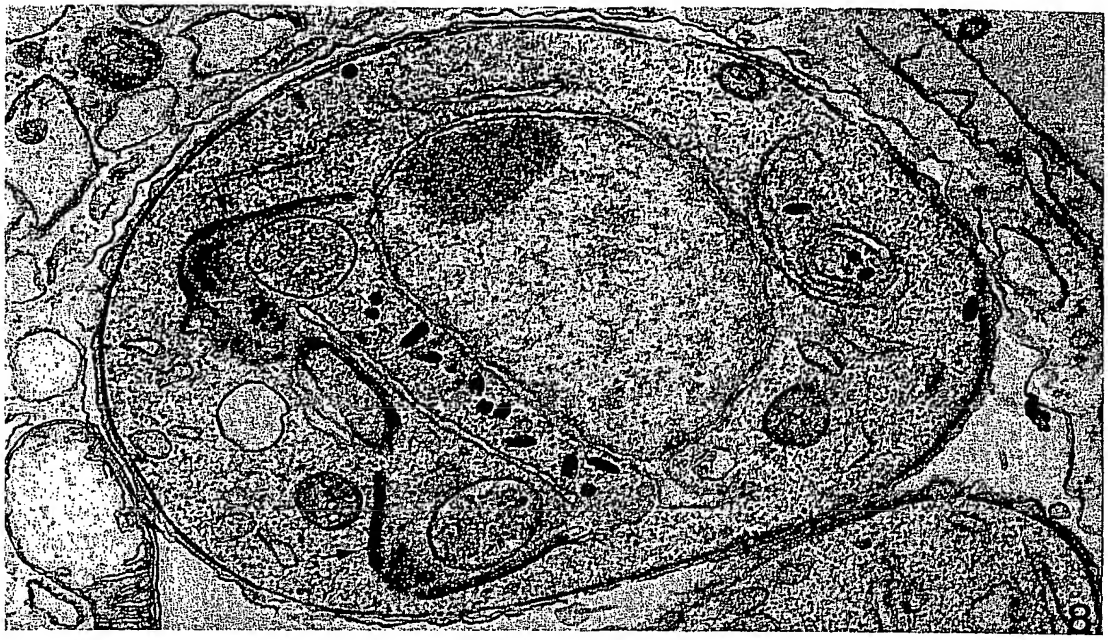
In most gregarines, the formation of the gametes proceeds similarly in gamonts of each sex. Therefore, the male and female gametes exhibit the same cytological features except for the occurrence of the undulipodium in the male. They are provided with the same cytoplasmic organelles (mitochondria, golgi, ER) and lipidic inclusions in the Stylocephalidae (Desportes, 1970).

The male gamete is motile owing to the undulipodium, which is more or less developed among gregarinian species (Grassé, 1953). The axoneme grows from the persistent polar kinetosome. In the gregarines and coccidia, the kinetosome is made up of nine singlet tubules instead of the triplets usually reported. Hematozoans bear standard kinetosomes.

The male gametes swim actively toward the female ones. Fertilization occurs in the cyst cavity and results in zygotes that undergo immediate meiosis and sporogenic processes.

b. Gamogony in coccidians:

Coccidian gamonts develop either from the extracellular trophonts, reported in the order Coelotrophiida, or from intracellular merozoites. In the genus *Cryptosporidium*, gamont development is thought to be extracytoplasmic but still intracellular. The extracellular gamonts tend to be larger than those lodged in host cells. The host cells are usually hyper-



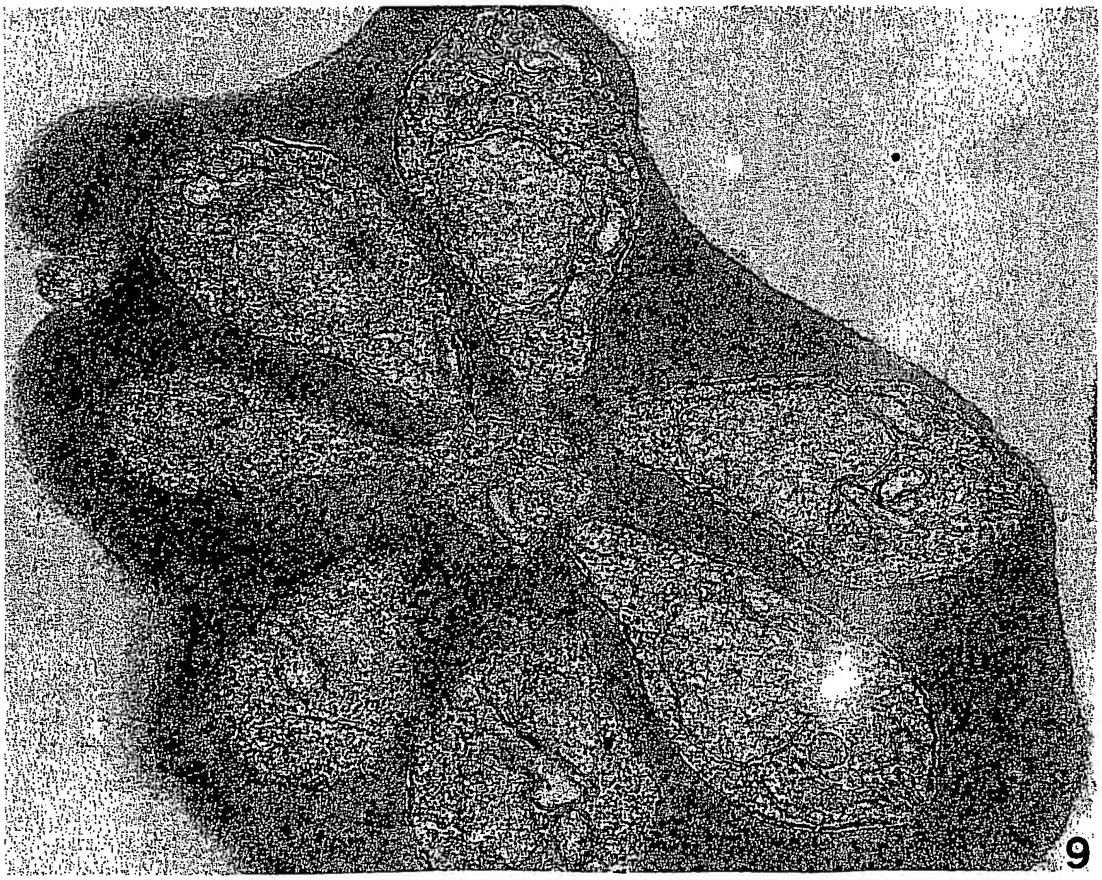


Fig. 8. The endogenesis in *Toxoplasma gondii*. The apical complexes of the two future merozoites (arrows) are already differentiated in the meront (Vivier, unpublished electron micrograph) × 26,000.

Fig. 9. The merozoites of *Anthemosoma garnhami* (Hematozoa, Piroplasmida) budding in a mouse red blood cell (Vivier and Petitprez, unpublished electron micrograph) × 20,000.

trophied owing to the relative enlargement of the parasite that enclosed in a parasitophorous vacuole (Fig. 4). The male gamonts are smaller than the female ones. They are therefore named the microgamont and macrogamont, respectively. Both gamonts differentiate in close association within the same cell in the order Adeleida. They develop in separate cells in Eimeriida.

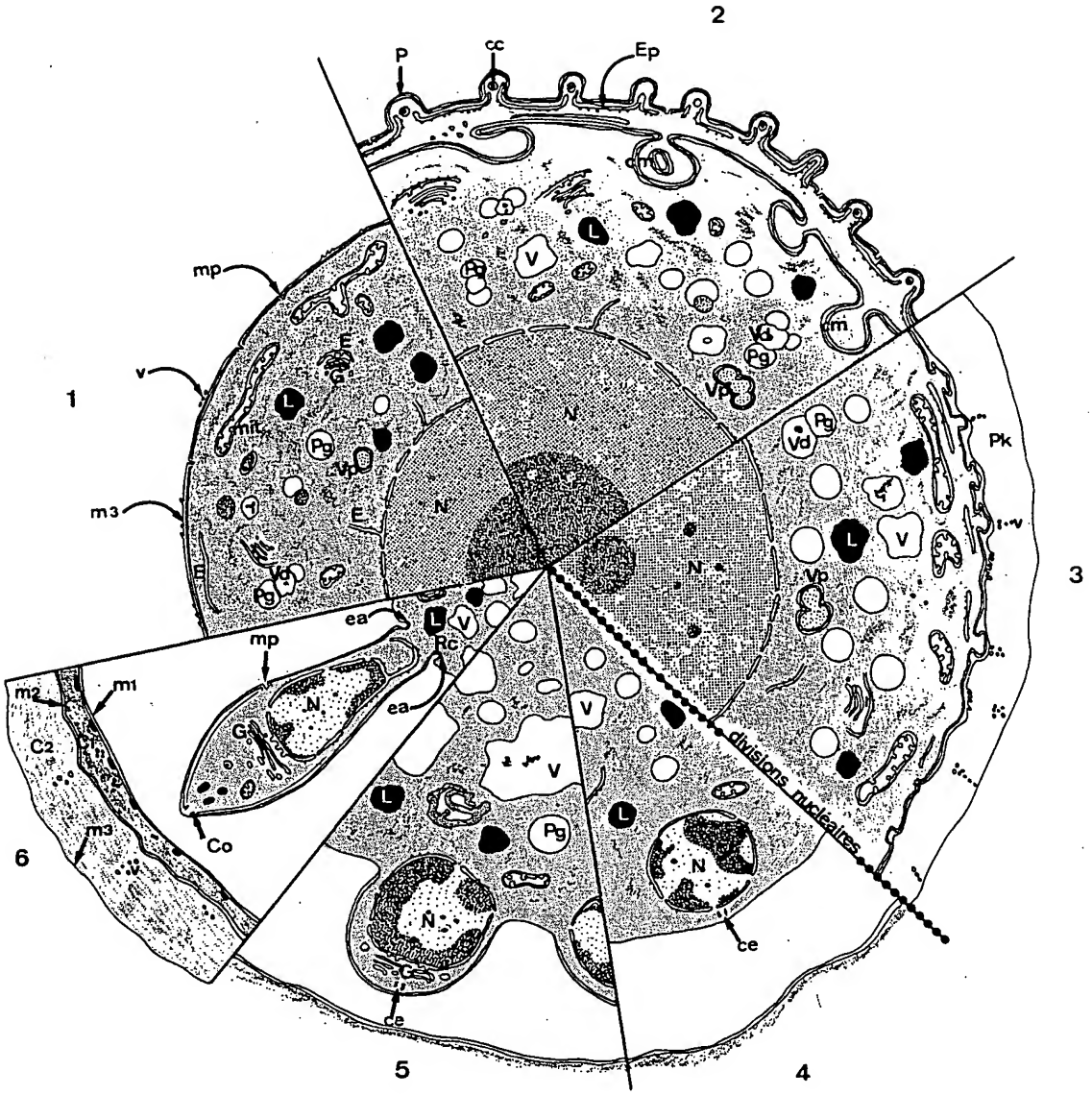


Fig. 10. The merogony in the coccidian *Aggregata eberthi*. 1, young meront with a smooth wall; 2, older meront with outer protrusions; 3, secretion of the cyst wall; 4, 5, 6, formation of the merozoite; C_1, C_2 mucous layers of the cyst wall; Ce, centriole; CM, cell membrane; Co, conoid; E, endoplasmic reticulum; ea, annular thickening of the stalk connecting the developing merozoite with the residual cytoplasm (Rc); G, golgi apparatus; L, lipids; mit, mitochondrion; 3m, membranes of the pellicle; mp, micropore; m1, m2, m3, membranes of the cyst wall; N, nucleus; P, protrusion of the meront wall; Pk, cyst wall; Pg, amylopectin granule; V, vacuole (from Porchet-Henneré and Richard, 1971).

In the two orders, the microgamont undergoes nuclear divisions giving rise to several to hundreds of microgametes whereas the macrogamont transforms into a single large macrogamete (see Scholtyseck in Hammond and Long, 1973).

Formation of the Microgametes. As in all gregarine gametes, the coccidian microgametes originate from the nuclei underlying the surface of the microgamont (Scholtyseck in Hammond and Long, 1973). However, only the dense part of the nuclear contents, a mitochondrion, and two polar kinetosomes are incorporated into the outgrowths budding at the surface of the gamont. These outgrowths detach, forming the microgametes. The microgametes are characterized by their small electron-dense nucleus, the occurrence of undulipodia, and of a single mitochondrion; other organelles and the light part of the nucleus are left in the residual part of the gamont (= residual body) (Fig. 18).

Microgametes are similar in all coccidians. They differ only in the number of undulipodia; two are reported in *Toxoplasma*, two or three in *Eimeria*, three in *Aggregata*.

The microgamete of the hematozoan *Plasmodium* bears only one undulipodium. Depending on the length of the undulipodia, the size of the microgametes average from 10 to about 30 μm.

The Macrogamete. The macrogamont transforms directly into a single macrogamete (Fig. 19). The latter retains most macrogamont features, such as a large nucleus and the occurrence of amylopectin granules. The cytoplasm in many coccidian genera contains peculiar inclusions that give rise to the oocyst wall after fertilization. These inclusions consist of the wall-forming

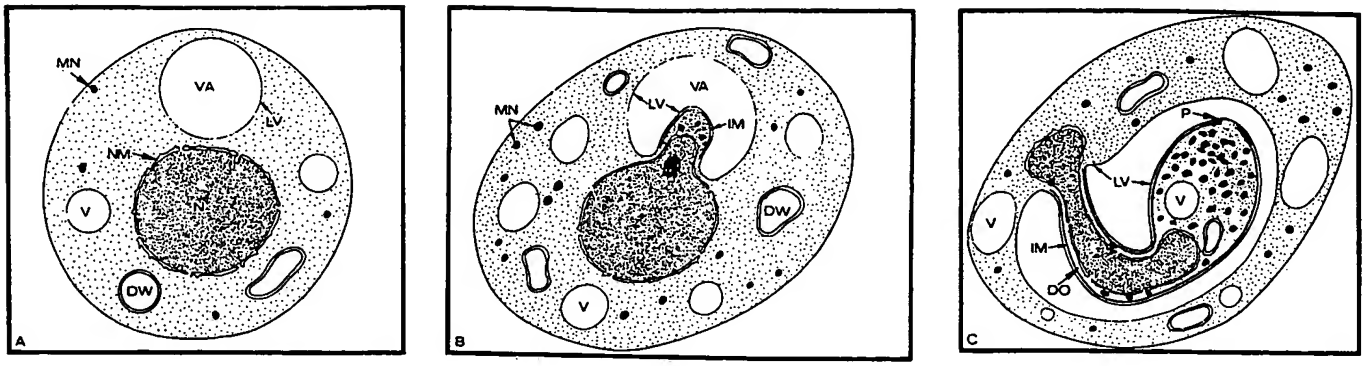
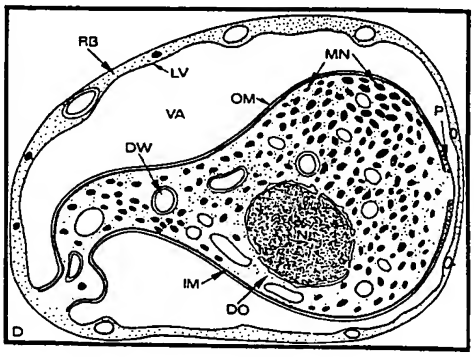


Fig. 11. Transformation of the spherical zygote of the hematozoan *Theileria* sp. (a) into a motile kinete (d). The latter protrudes (b, c) in a vacuole previously differentiated in the zygote cytoplasm. DO, kinete; DW, mitochondrion; IM, OM, inner and outer membranes of the pellicle; LV, limiting membrane of the vacuole giving rise to the outer membrane of the pellicle; MN, micronemes; NM, nuclear envelope; N, nucleus; P, polar ring; RB, residual part of the kinete; V, VA, vacuole (from Mehlhorn *et al.*, 1979).



bodies I and II. The former are granules more or less dense whereas the latter frequently exhibit a spongelike appearance, as observed in the Eimeriidae (Fig. 19). Wall-forming bodies are lacking in the coccidian macrogametes, which differentiate a thin cyst wall as reported in *Klossia* and *Aggregata*.

After fertilization, the macrogamete becomes an oocyst. In *Eimeria* and *Toxoplasma*, the wall-forming I bodies are then exuded in order to form the outer layer of the oocyst wall whereas the wall-forming II bodies constitute the inner layer (Fig. 19). For details see Scholtyseck in Hammond and Long, 1973.

In the hematozoan order Haemosporida, gamogony is similar to that reported in the Coccidia. The microgamonts and macrogamonts of *Plasmodia* differentiate from merozoites lodged in the red blood cells of vertebrates (Reptilia, Aves, Mammalia). Red cells containing gamonts are ingested by mosquitoes that digest the cells blood. The gamonts, which transform into microgametes and macrogametes, are released in the insect digestive system. Fertilization occurs in the midgut or the stomach and results in the formation of a motile zygote, the kinete or ookinete, which exhibits the apical complex and pellicular organization of a zoite (Fig. 11).

Gamogony proceeds differently in piroplasms. In *Babesia*, the gamonts produce a small number of gametes characterized by the occurrence of cytoplasmic arms and a tail containing microtubules. A second type of gamete is equipped with an arrowhead structure. Fertilization consists of the fusion of gametes of each type.

The resulting zygote contains the arrowhead organelle, which may play a role in the penetration of the gut wall of ticks where the sporogenic processes begin (Rudzinska *et al.*, 1984).

The Sporogenic Phase or Sporogony

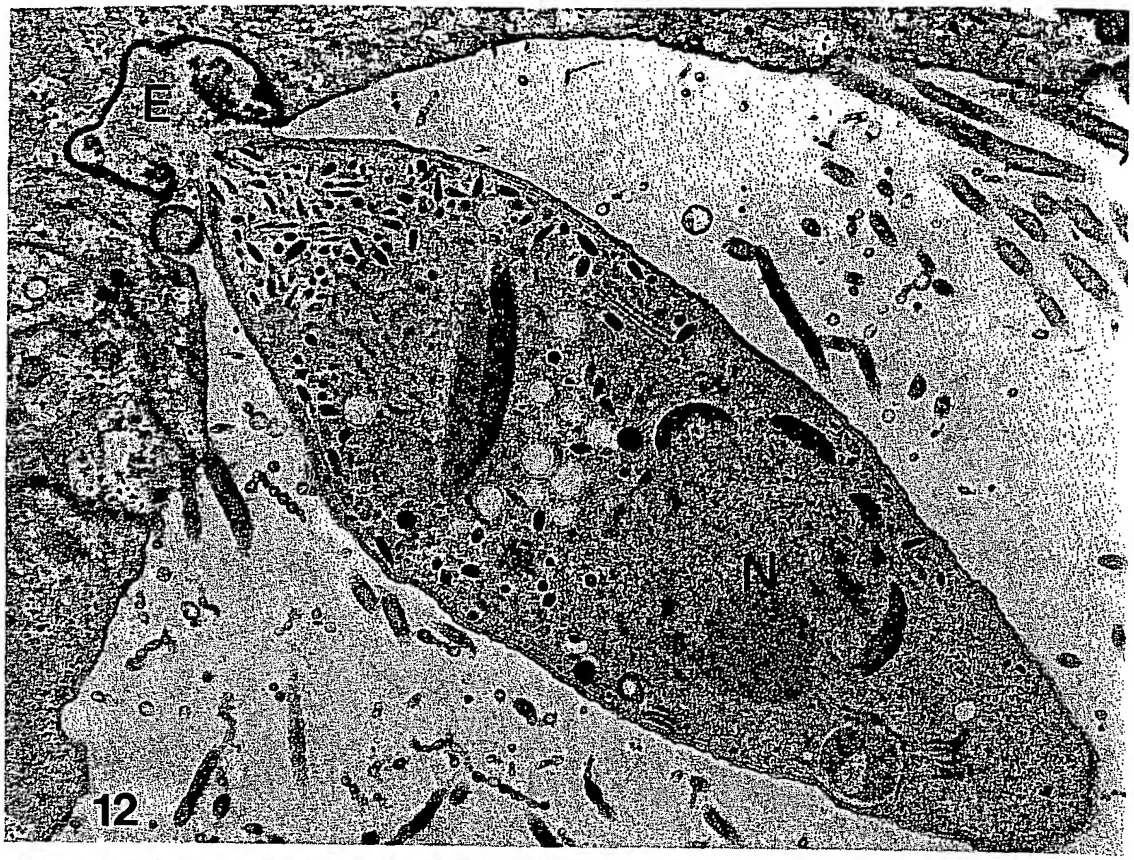
Sporogony consists of the production of haploid sporozoites by the zygote. The first divisions of the zygote are meiotic. They usually occur soon after fertilization as confirmed in the gregarine *Grebnickiella gracilis* (Molon-Noblot and Desportes, 1977). Except for the zygote stage, the Apicomplexa are haploid during their life cycle.

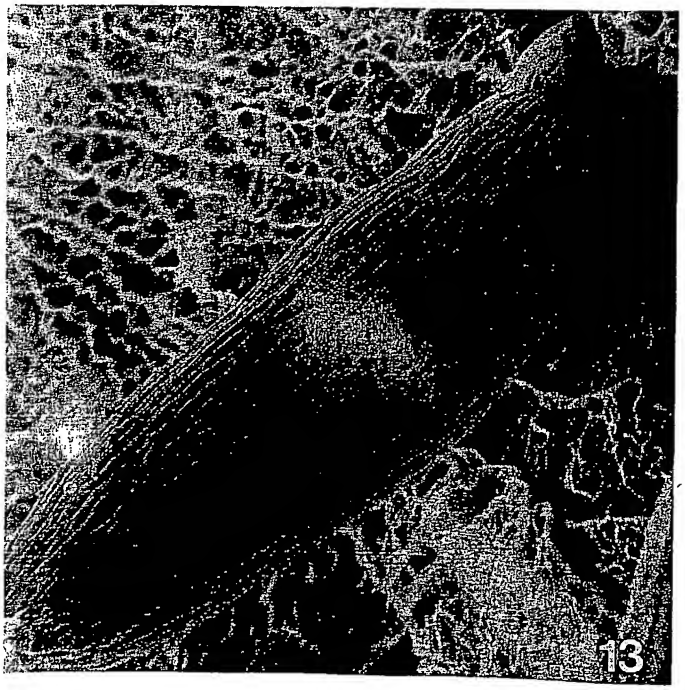
There are differences in sporogony among the apicomplexan classes, gregarines, coccidians, and hematozoans.

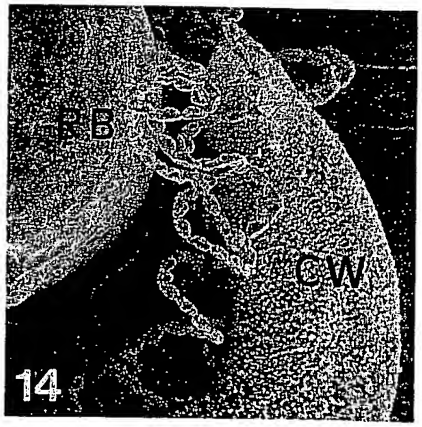
The gregarine zygote undergoes meiosis and secretes a spore wall (Figs. 14, 15, 16). Eight cells with the characters of zoites (= sporozoites) are usually produced. In all gregarines, except for the order Blastogregarinida, the sporogony occurs in the cyst wall previously secreted by the paired gamonts. The mature sporocysts are released either by rupture of the cyst wall (Figs. 14, 15) or by tubular expansions called sporoducts. The latter are differentiated in some families: Gregarinidae, Gigaductidae, Monoductidae (see Table 1). The sporogony of intestinal gregarines begins in the lumen of the gut. The maturation of the sporocysts is usually achieved when the cysts are ejected with host feces into the external environment. The sporogony of hemocoelian gregarines (Urosporidae, Monocystidae, Diplocystidae) occurs in the body cavity and the sporocysts are disseminated after the death of the host.

In the Blastogregarinida, a "primitive" group described by Chatton and Villeneuve (in Grassé, 1953), gamogony and sporogony proceed without preliminary pairing and encystment. The gametes are therefore released directly into the lumen of the gut where fertilization and subsequent sporogony occur.

A great number of sporocysts are usually produced owing to the quantity of gametes originating from large extracellular gamonts. The number of sporocysts and gametes is lower when the gamonts develop within cells or tissues as in the order Neogregarinida.







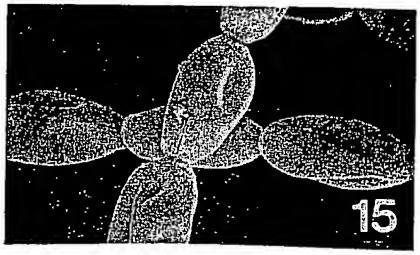


Fig. 12. The sporozoite of the gregarine *Stylocephalus* sp. differentiating an epimerite (E) in the intestinal epithelium of its host; N, nucleus (from Desportes, 1969) × 32,000.

Fig. 13. Scanning electron micrograph of the growing gamont (= trophozoite) of *Stylocephalus* sp. The longitudinal pellicular folds are visible (from Desportes, 1975) × 1,020.

Figs. 14–15. Scanning electron micrographs of a sporulating cyst and spores of *Stylocephalus* sp. RB, residual body; CW, cyst wall. As in many gregarines, the spores are arranged in a chain (from Desportes, 1975) × 250; 1,725

In coccidians sporogony also results in the production of sporocysts containing sporozoites. However, the coccidian sporocysts do not exactly correspond to gregarine sporocysts, which are zygotes transformed by the sporogenic processes through wall secretion and sporozoite production.

In coccidians, the zygote is the fertilized macrogamete that is enclosed by the resistant outer covering produced by the wall-forming bodies, the oocyst wall. The zygote divides into cells called sporoblasts that, like the gregarine zygotes, transform into sporocysts; they then secrete a spore wall and undergo divisions resulting in the formation of sporozoites (Fig. 22). The number of sporocysts in the oocyst and the quantity of sporozoites in the sporocysts are most significant as regards the classification of Coccidia. Furthermore, in some groups, such as the Cryptosporidae and the Lankesterellidae, the sporozoites are differentiated without the concomitant secretion of a spore wall.

The intestinal oocysts are ejected with the host feces. New hosts will be infected by the ingestion of mature oocysts or sporocysts (Fig. 22).

In hematozoans, as in the coccidians, the zygote corresponds to the fertilized macrogamete. The fertilization occurs in the gut of a blood-sucking invertebrate. The zygote does not transform immediately into an oocyst that may be ejected with host feces as previously reported. It develops into a motile stage, the ookinete (Fig. 11), able to penetrate the stomach wall as observed in *Plasmodia* (see reviews published in Kreier, 1977). The ookinete rounds up and secretes an envelope, transforming into an oocyst. A large number of naked zoites are differentiated in the oocyst by a process similar to that reported in merogony. The zoites are released in the body cavity and migrate toward the salivary glands of the host, from which they will be inoculated into a vertebrate.

In piroplasms, the kinetes migrate to the salivary glands and penetrate them. The differentiation of the zoites occurs there. In *Theileria,* more than 100,000 zoites may be produced by each kinete and then transformed into sporonts (Mehlhorn *et al.*, 1979).

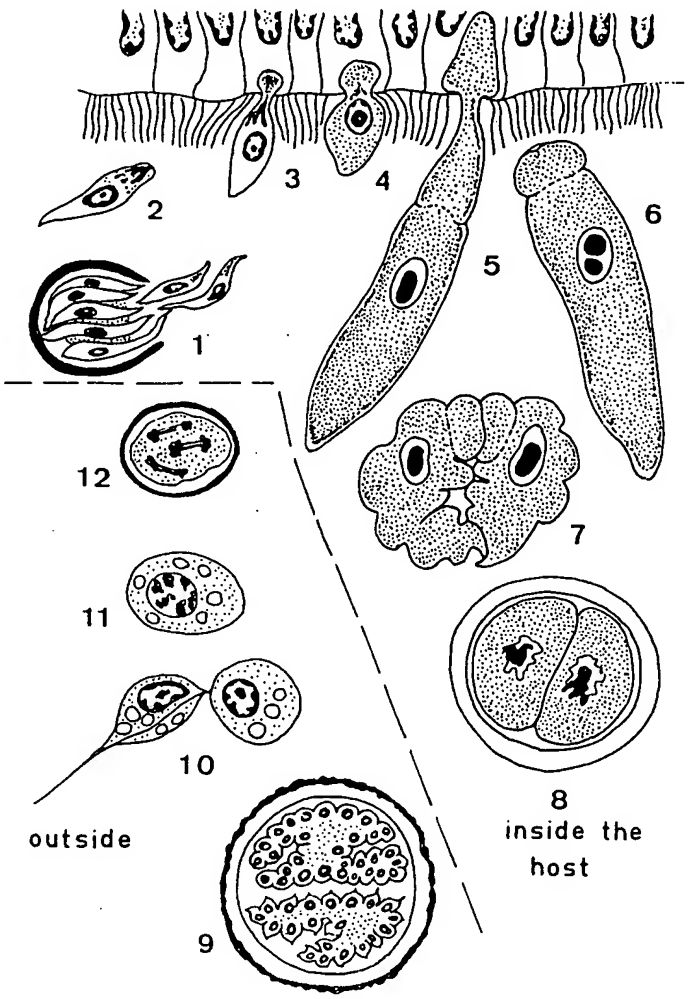


Fig. 16. Life cycle of the gregarine *Stylocephalus* sp. 1–8, developmental stages occurring in the intestine of the host (a tenebrionid beetle): 1, sporozoite escaping from the spore ingested by the host; 2, free sporozoite; 3, differentiation of the epimerite; 4–5, growing gamonts (= trophozoites); 6, mature gamont detached from the intestinal epithelium; 7, pairing of gamonts; 8, encystment. 9–12, developmental stages occurring outside the host: 9, differentiation of gametes (female gametes in the upper gamont, male ones in the lower); 10, fertilization; 11, the zygote; 12, meiosis (10, 11, and 12 occur under the cyst wall).

Methods of Recognition

The extracellular stages (trophonts and cysts) of gregarines and coccidians may be easily observed *in vivo* in the gut or in the body cavity of crustaceans and insects. They are recognizable at lower magnifications with binocular microscopes owing to their relatively large size (about 200 to 700 μm, frequently several millimeters for gregarine trophonts). The trophonts are rather spherical in coccidians, most often elongate in gregarines. Furthermore, older trophonts detached from the gut wall are motile in the latter group. The nucleus is spherical or ellipsoidal and looks slightly darker than the clear cytoplasm.

Mature cysts are yellow or brown, depending on the thickness of the envelope.

Classical techniques of light microscopy are of course more informative with regard to cytological features and the identification of smaller stages, i.e., sporocysts, zoites, or gametes, which average from 1.5 to about 20 μm. However, the use of the electron microscope has been important in defining the phylum and in the identification of its members. The ultrastructural characters of the zoites may be considered to be the most significant criterion in the diagnosis. For example, the piroplasmids and the toxoplasms, which were considered to be groups of uncertain position, were included in the phylum after the demonstration of the apical complex in their infecting stages (Fig. 3).

Classification

The Apicomplexa are subdivided into three classes: Gregarinia, Coccidia, and Hematozoa (Vivier, 1982).

The gregarines and coccidians differ essentially in their gamogony (female gamont giving rise to a number of gametes in

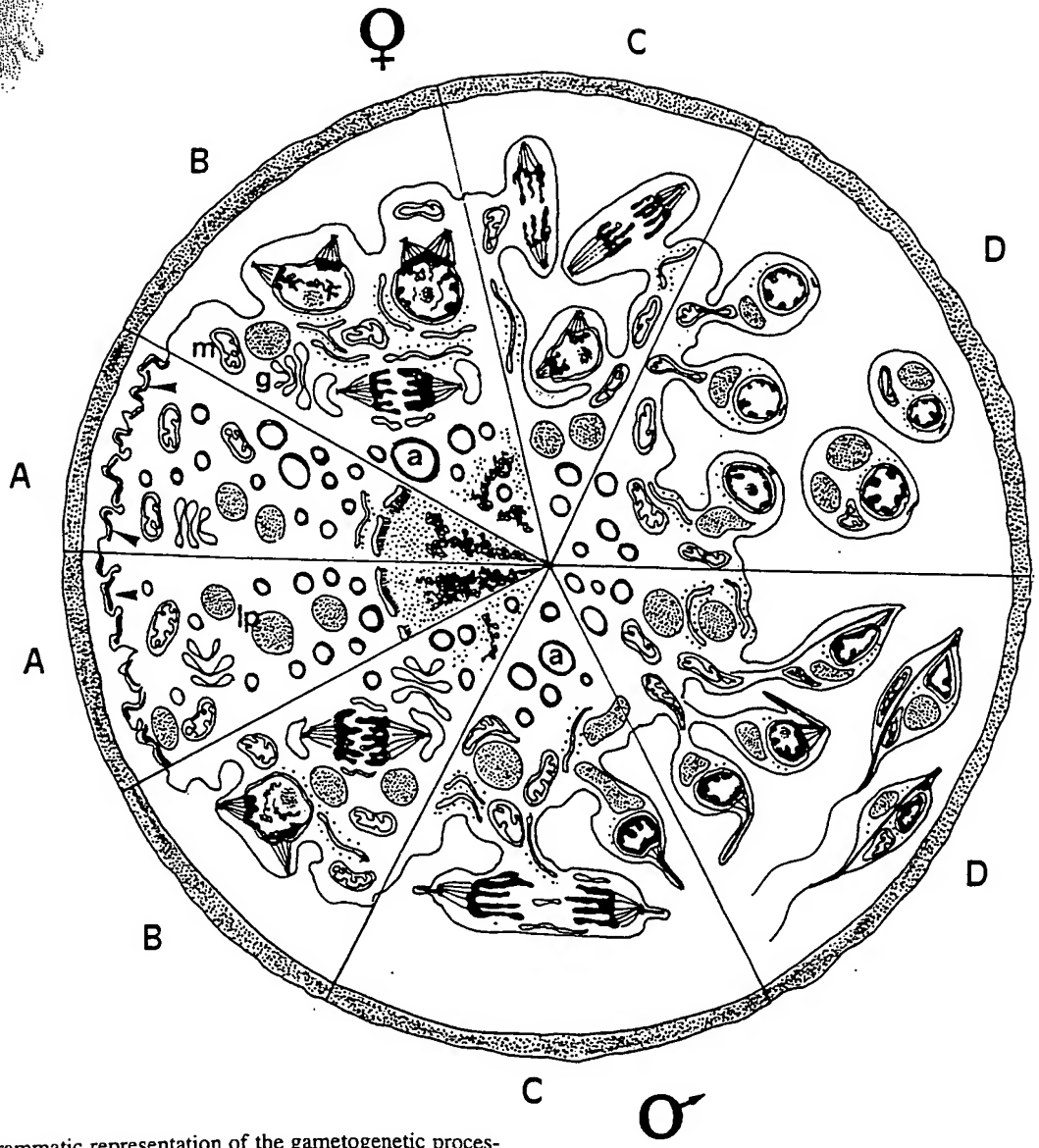


Fig. 17. Diagrammatic representation of the gametogenetic processes in female (upper) and male (lower) gamonts of the gregarine *Stylocephalus* sp. a. the gamonts after the encystment. The inner membranes of the pellicle are disorganizing (arrowheads); b. gamogonic nuclear divisions; c. differentiation of the gametic nuclei. Those of the future male gametes are recognizable by the undulipodium arising from the polar centriole; d. budding of the mature gametes in the cyst cavity. All sequences occur within the common cyst wall secreted by both gamonts. a, amylopectin; lp, lipids; g, golgi; m, mitochondrion (Desportes, original).

the former, to a single one in the latter). The gamogony of the hematozoans is of the coccidian type. They are considered to be a distinct order owing to peculiarities of their sporogony and cytological features (Vivier, 1982). The orders and principal families are listed below. All taxa prior to 1953 are detailed in Grassé (1953).

Class GREGARINIA Dufour, 1828

The Gregarinia are characterized by the production of an equal quantity of gametes by male and female gamonts. The zygote transforms directly into a sporocyst giving rise to sporozoites.

They are all monoxenic parasites in invertebrates. The most "primitive" groups develop in marine hosts (Théodoridès, 1984). The class comprises four orders:

Order 1. Blastogregarinida Chatton and Villeneuve, 1936

Characters of the order: Extracellular trophonts transform directly into gamonts attached to the gut wall of the host. The gamonts undergo gamogony separately. The sporocysts contain 10 to 16 sporozoites. One species, parasitic on a marine annelid.

Order 2. Archigregarinida Grassé, 1953

Characters of the order: Intestinal trophonts characterized by the persistence of zoite organelles (Fig. 20), pairing and encystment of the gamonts, and sporocysts containing 4, 8 or more zoites, according to the genera.

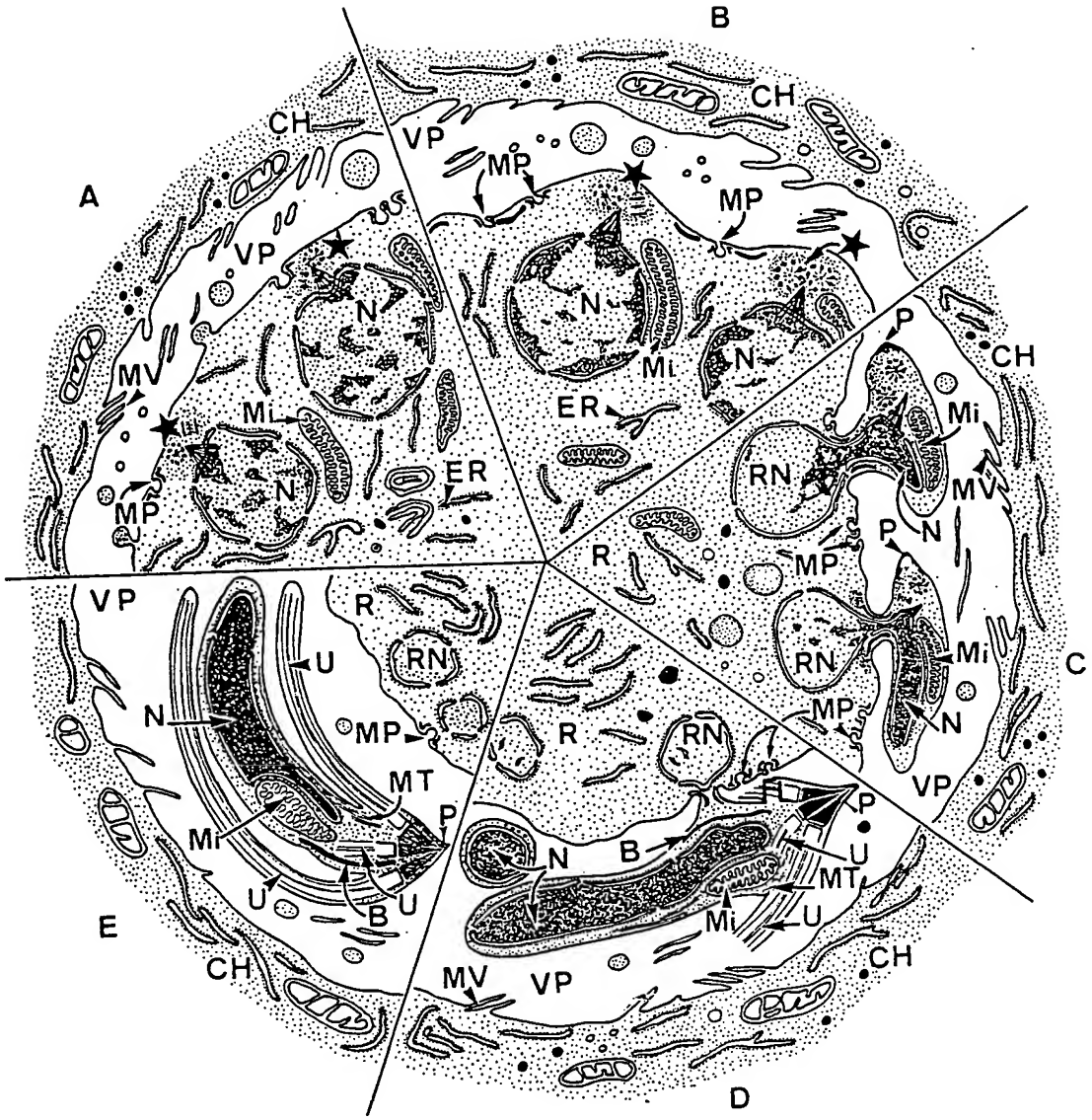


Fig. 18. The differentiation of the microgametes in the coccidian *Eimeria acevulina*. a. microgamont with gametic nuclei; b. condensation of the chromatin; c. incorporation of the denser part of the nucleus in the budding microgamete; d. the nearly mature microgamete still connected with the residual body; e. the mature microgamete. B, dense rod; CH, host cell; ER, endoplasmic reticulum; Mi, mitochondrion; MP, micropore; MT, microtubules; MV, microvilli; N, nucleus; P, perforatorium; R, residuum; RN, nuclear residuum; U, undulipodium; VP, parasitophorous vacuole (from Senaud *et al.*, 1980).

Two families: Selenidiidae Brasil, 1907 with four genera parasitic on marine worms, and Merogregarinidae Fantham, 1908 with one genus and species developing in ascidians.

Order 3. Eugregarinida Léger, 1899?

Characters of the order: Extracellular development of trophonts, pairing and encystment of gamonts, sporocysts containing 8 sporozoites.

The order is represented by a great number of genera and species. About 18 families are recorded: 8 parasitic on marine invertebrates, 11 on terrestrial and freshwater invertebrates.

Their classification is based upon:

—the location in the host (gut or body cavity)

—the phyletic position of the host

—the process of development (solitary or in pairs) and cytological features of the trophonts (occurrence and shape of the adhering apparatus, trophont subdivided or not by a fibrillar septum) (Fig. 21).

—the type of sporulation (rupture of the cyst wall or sporoducts) and the shape of the sporocysts.

The families are listed in Table 1.

Order 4. Neogregarinida, Grassé 1953

Characters of the order: Intracellular development in tissue. Small gamonts give rise to a small number of gametes. A small number of sporocysts contain 8 sporozoites. Merogony occurs in the trophic phase. The order comprises about 6 families parasitic on insects. The Lipotrophiidae are pathogenic.

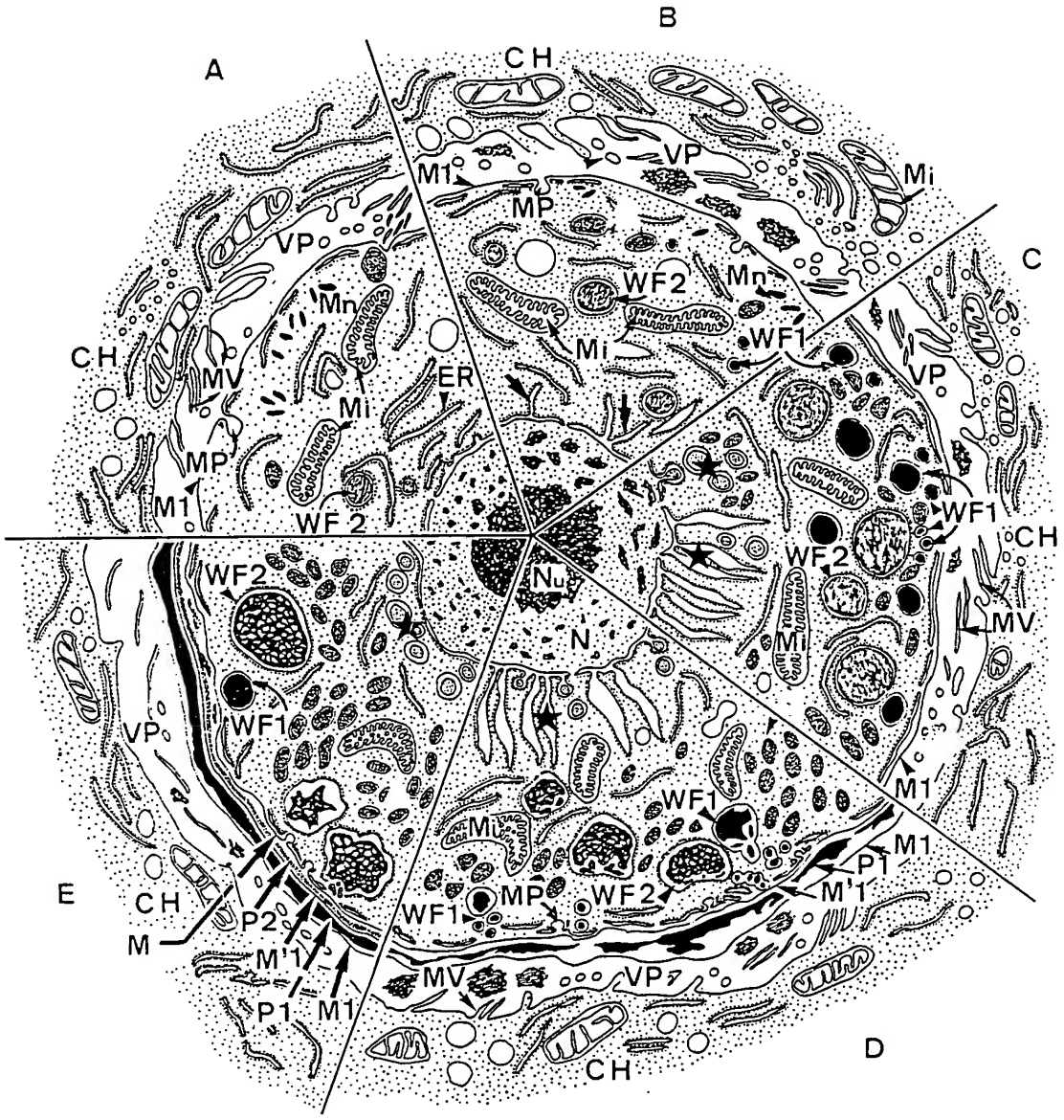


Fig. 19. Transformation of the macrogamont of the coccidian *Eimeria acervulina* into an oocyst. a., b. formation of the wall-forming bodies (WF1, WF2) in maturing macrogamont; c. macrogamete characterized by evaginations of the nuclear envelope; d. the fertilized macrogamete begins to secrete the oocyst wall; e. the oocyst. CH, host cell; ER, endoplasmic reticulum; M, M1, M'1, membranes of the cyst wall; Mi, mitochondrion; Mn, micronemes; MP, micropore; MV, microvilli; N, nucleus; Nu, nucleolus; VP, parasitophorous vacuole (from Senaud *et al.*, 1980).

Class COCCIDIA Leuckart, 1879

Gamogony is characterized by the development of the female gamont into a single macrogamete. The zygote is the oocyst, which divides into sporoblasts. The latter give rise to sporozoites usually enveloped by a sporocyst wall secreted by the sporoblast. Coccidians are parasitic on invertebrates and vertebrates. Some groups are heteroxenous; their development requires two successive hosts. According to Vivier (1982), the class is subdivided into three orders: Coelotrophiida, Adeleida, and Eimeriida.

Order 1. Coelotrophiida Vivier 1982

Characters of the order: Extracellular trophic phase without merogony, extracellular gamonts. Five families are parasitic on marine annelids. Four develop in the body cavity: Coelotrophiidae Vivier, 1981, Angeiocystidae Léger, 1911, Myriosporidae Grassé, 1953, and Mackinnoniidae Vivier, 1981. The intestinal Eleutheroschizonidae Chatton et Villeneuve are placed in the order because of their extracellular development.

Order 2. Adeleida, Léger, 1911

Characters of the order: Male and female gamonts are closely associated during their development. Microgamonts give rise to a small quantity of gametes. Merogony occurs in the growth phase. There are 4 homoxenous families: 1) Adeleidae Mesnil,

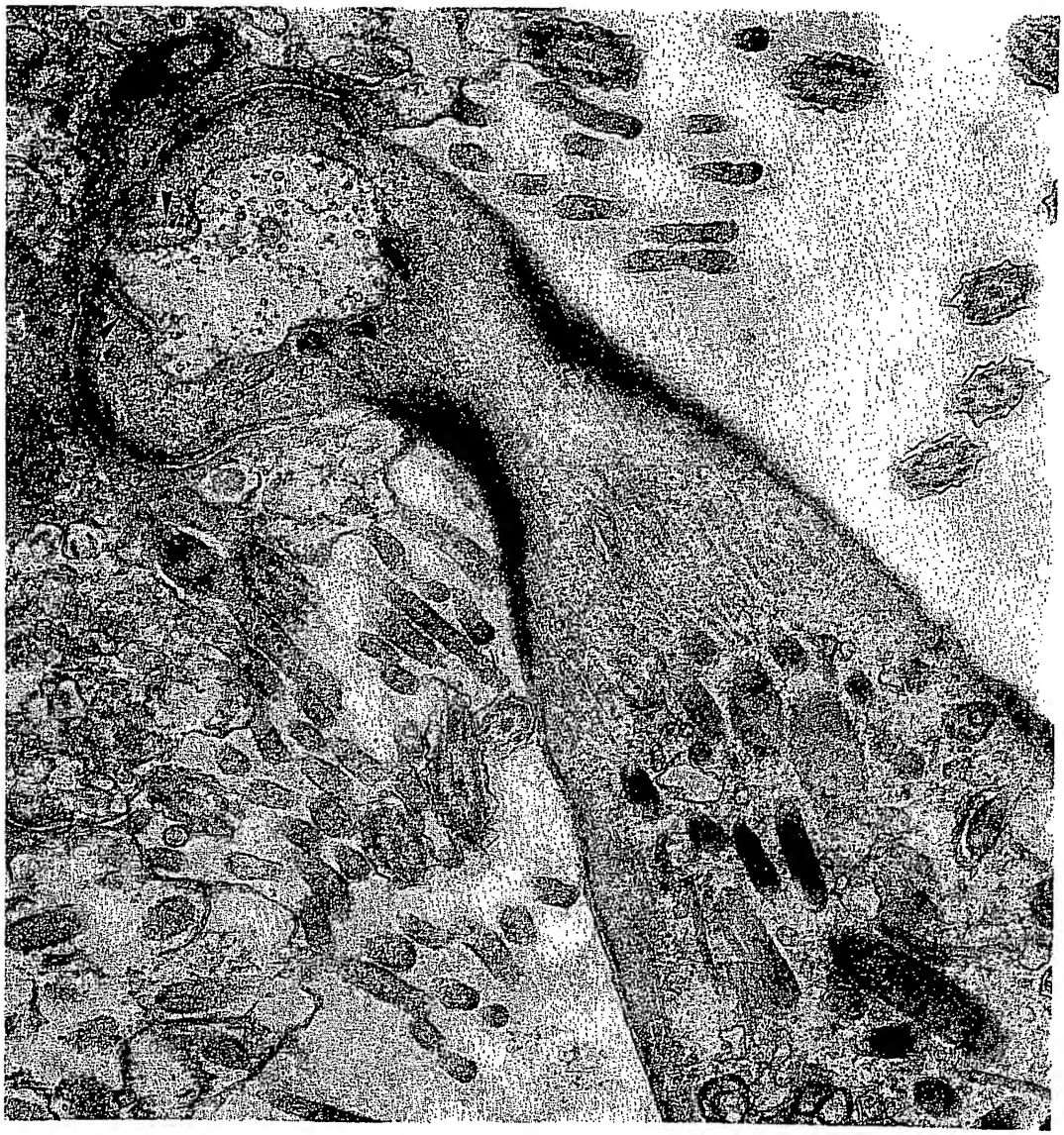


Fig. 20. The anterior part of a young trophozoite of the gregarine *Selenidium hollandei*. Sections of the conoid can be seen (arrows) in the apical part. The latter expands in an invagination of the host's intestinal cell thus forming an anchoring apparatus. The occurrence of the anterior vacuole is related to feeding processes (from Schrevel, 1968) × .000.

1905, parasitic in Chilopoda, Oligochaeta, and Insecta; 2) Klossiidae Grassé, 1953, developing in Mollusca and Hirudinea; 3) Legerellidae Minchin, 1905, in which the oocysts give rise to naked zoites, parasitic on Diplopoda and Insecta; and 4) Dobelliidae Ikeda, 1914, parasitic on Sipunculida. These are considered to link Adeleida and Eimeriida owing to the numerous microgametes produced by the microgamont.

The Haemogregarinidae Léger, 1911, develop in two successive hosts: a vertebrate and a blood-sucking invertebrate. For example, merogony and differentiation of the gamonts of *Haemogregarina stepanovi* occur in a terrapin. The gamonts are ingested by a leech. The gametes are released in the gut of the leech and fertilization results in an oocyst. Further sporogenic processes are similiar to those observed in Hematozoa: the naked zoites formed in the oocyst cross the gut wall and reach the trunk of the leech, from which they may be inoculated into other terrapins.

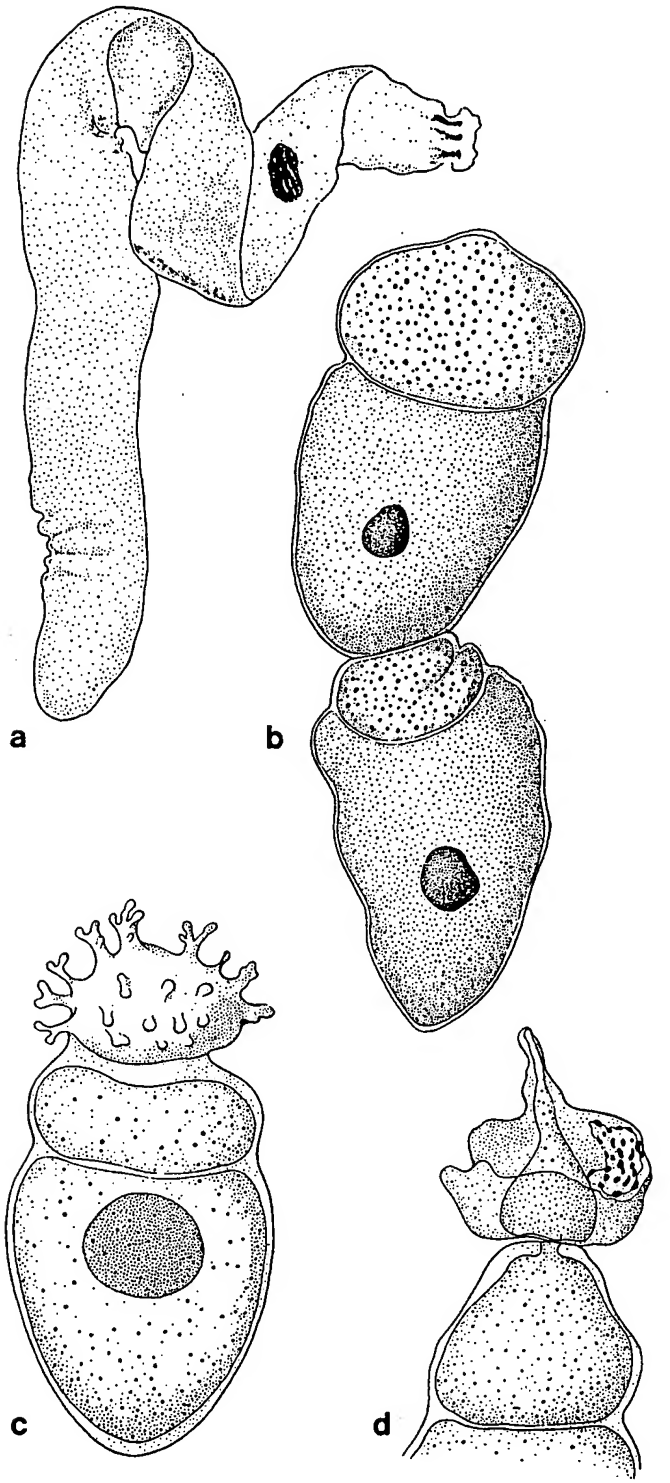
Order 3. Eimeriida Léger, 1911

Characters of the order: Male and female gamonts develop separately, production of a high quantity of microgametes, parasitic on invertebrates and vertebrates. The classification is usually based upon the number of sporozoites produced by the oocyst and the presence or absence of a spore wall (see Grassé, 1953, and Levine in Hammond and Long, 1973).

Of the approximately 19 families, 17 are homoxenous, and 2 are heteroxenous.

Homoxenous families:

1. Cryptosporidae Léger, 1911: Extracellular development of



gamonts; oocyst giving rise to 4 naked zoites; parasitic in mammals.

2. Mantonellidae Grassé, 1953: Intracellular development; one sporocyst per oocyst; 4 sporozoites, parasitic on invertebrates.

3. Cyclosporidae Léger, 1911: Oocyst containing 2 sporocysts with 2 sporozoites; parasitic on mammals.

4. Pfeifferinellidae Grassé, 1953: Oocyst with 8 naked zoites. Fertilization of the macrogamete through a "vaginal tube." Parasitic on molluscs.

5. Caryosporidae Léger, 1911: Oocyst containing sporocysts with 8 sporozoites, parasitic on Ophidia and birds.

6. Diplosporidae Léger, 1911: Oocyst producing 2 sporocysts with 4 sporozoites; parasitic on molluscs, frogs, reptiles, birds, and mammals.

7. Eimeriidae Minchin, 1903: Intracellular development, 4 sporocysts with 2 sporozoites per oocyst. Many species are pathogenic owing to the occurrence of several generations of merozoites destroying the intestinal cells of the host. *Eimeria cyprinorum* and *E. carpelli* are parasitic on fish. *E. tenella, E. maxima, E. necatrix, E. truncata, E. phasiani* are the agents of severe infections called coccidiosis in domestic birds (see Ruff and Reid in Kreier, 1977). *E. perforans, E. stiedae, E. irresidua, E. magna* are pathogenic in the domestic rabbit. *E. zurmii* and *E. bovis* are pathogenic in Bovidae.

8. Dorisiellidae Grassé, 1953: 2 sporocysts with 8 sporozoites per oocyst; parasitic in marine worms (Polychaeta).

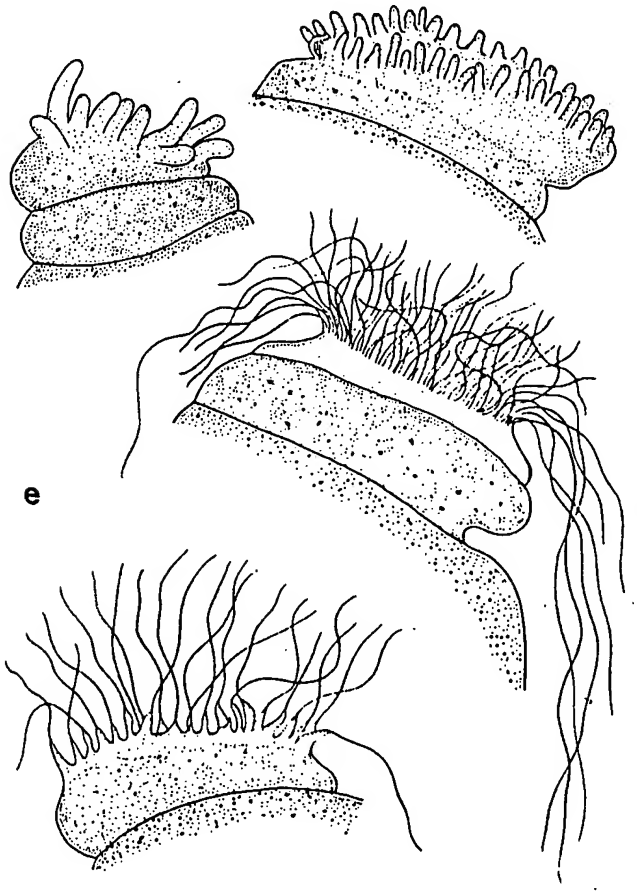


Fig. 21. Gregarine trophozoites. a. the ribbonlike trophozoite of *Ganymedes* sp. (from Théodoridès and Desportes, 1975); b. two septate trophozoites of *Gregarinia* sp. associated according to the caudofrontal arrangement; c. the young septate trophozoite of *Ramicephalus* sp. (Actinocephalidae). Its globular epimerite is ornamented with ramified appendages; d. the anterior part of a young trophozoite of *Gregarinia* sp. The apical epimerite is still enclosed in remnants of the host cell (from Théodoridès *et al.*, 1972); e. different aspects of the anchoring apparatus in four species of *Echinomera* (Dactylophoridae) (from Ormières, 1966). Length of trophozoites average 500 μm in *Ganymedes* (a), 100 μm in *Gregarinia* (b).

9. Wenyonellidae Grassé, 1953: 4 sporocysts with 4 sporozoites per oocyst; parasitic in reptiles, birds, and mammals.

10. Barrouxiidae Léger, 1911: Production of a large number of sporocysts with one sporozoite per oocyst. Parasitic in insects and Chilopoda.

11. Caryotrophidae Luhe, 1906: Oocysts containing an undetermined number of sporocysts with 12 sporozoites; parasitic in polychaetes.

12. Lankesterellidae Noller, 1920: Oocyst producing from 12 to 50 naked zoites. All developmental sequences occur in frogs but the sporozoites are transmitted by leeches.

13. Yamikovellidae Goussef, 1937: 8 sporocysts containing a large number of sporozoites per oocyst; parasitic in mammals.

Four other families mentioned by Grassé (1953) are only known by their gamogony and sporogony: Angeiocystidae Léger, 1911, Psyedoklossiidae Grassé, 1953, Myriosporidae Grassé, 1953, and Merocystidae Grassé, 1953. All are parasitic in invertebrates.

Heteroxenous families:

1. Aggregatidae Labbé, 1899: Life cycle completed in two invertebrates. Gamogony and sporogony occur in Cephalopoda (Mollusca) and merogony in Decapoda (Crustacea). The oocyst contains a number of sporocysts with a specific number of sporozoites.

2. Sarcocystidae Poche, 1913: Life cycle completed in two mammals. Two subfamilies according to Frenkel (1977): Sarcocystinae Poche, 1913 and Toxoplasmatinae Biocca, 1956. Gamogony and sporogony occur in the gut of a carnivorous predator, and merogony occurs in an intermediate host, which is usually a prey species. The intermediate host is infected by the ingestion of mature sporocysts. The meronts encyst within the muscles.

Sarcocystis suihominis (Fig. 22), *S. bovicanis*, and *S. ovicanis* are pathogenic for the intermediate host.

Toxoplasma gondii is responsible for alterations of the nervous system and human fetal malformations.

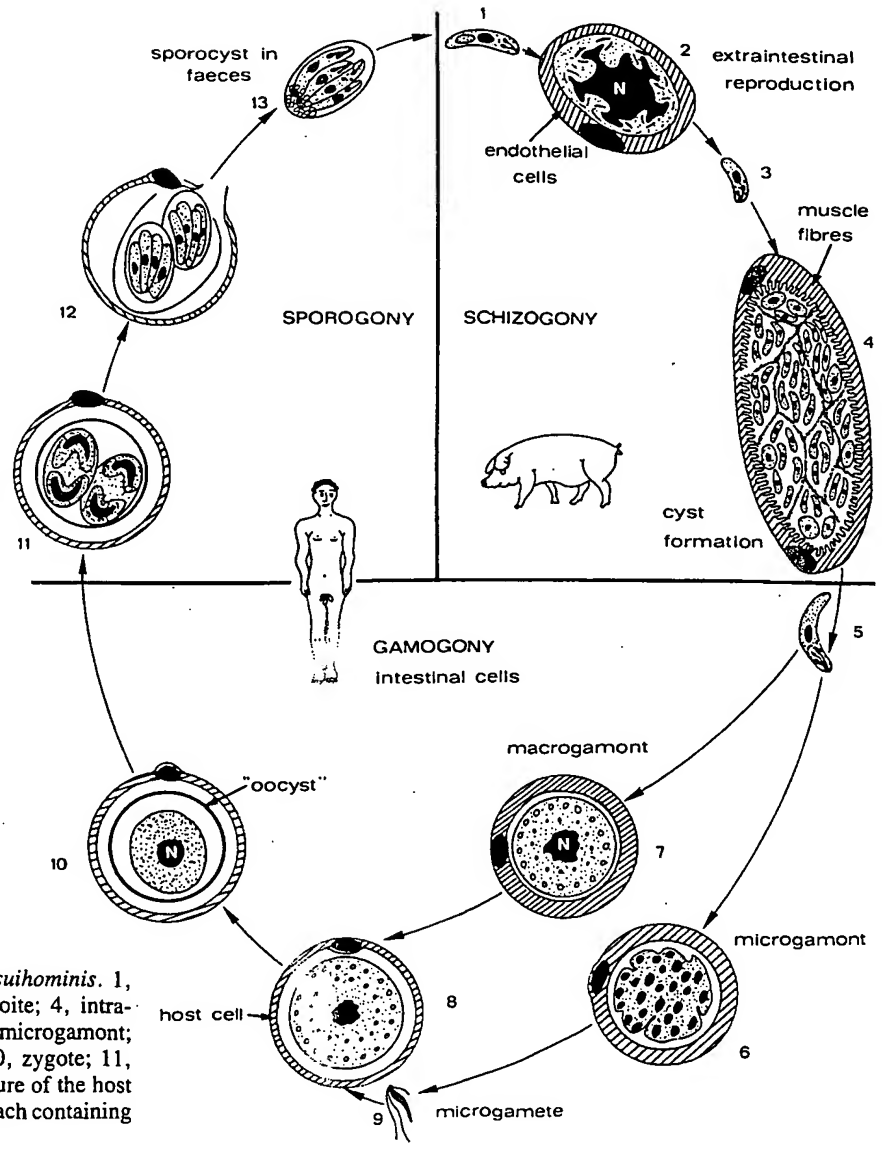


Fig. 22. The life cycle of the coccidian *Sarcocystis suihominis*. 1, sporozoite; 2, meront with a large nucleus; 3, merozoite; 4, intramuscular encysted meront; 5, intracystic merozoite; 6, microgamont; 7, macrogamont; 8, macrogamete; 9, microgamete; 10, zygote; 11, differentiation of two sporocysts in the oocyst; 12, rupture of the host cell and of the oocyst wall releasing the two sporocysts each containing four sporozoites. (from Mehlhorn *et al.*, 1979).

Class HEMATOZOA Vivier, 1982

The class is characterized by zoites with a rudimentary apical complex (without conoid and conoidal rings). Motile zygotes (= kinete) penetrate the gut wall and give rise to naked zoites. The life cycle is heteroxenous: merogony and formation of gamonts occur in the blood of a vertebrate; the maturation of gametes, fertilization, and sporogony occur in hematophagous invertebrates.

Order 1. Haemosporida, Danilewsky, 1885

Characters of the order: Merogony and the beginning of gamogony in the blood system of vertebrates; production of gametes, fertilization, and sporogony in blood-sucking dipteran flies.

Haemoproteus is parasitic in reptiles and birds, *Leucocytozoon* develops in birds, and *Hepatocystis* in mammals (see Fallis and Desser in Kreier, 1977). *Plasmodium* species are reported in reptiles (see Ayala in Kreier, 1977), in birds (Seed and Manwell in Kreier, 1977) and mammals, especially rodents (Carter and Diggs in Kreier, 1977), and primates (Collins and Aikawa, Rieckmann and Silverman in Kreier, 1977).

Four *Plasmodium* species are pathogenic to humans owing to the destruction of blood cells by generations of merozoites and the subsequent releasing of antigen. Other tissues are also infected (exoerythrocytotic development).

Order 2. Piroplasmida Wenyon, 1936

Characters of the order: Gamogony and sporogony in ticks (Ixodidae), merogony in mammals. *Babesia* is the agent of piroplasmosis. *B. canis* is pathogenic for dogs. *Theileria* is the agent of theileriosis in cattle (see reviews of Mahoney, Ristic and Lewis, Barnett in Kreier, 1977).

MAINTENANCE AND CULTIVATION

Apicomplexa are collected in nature from parasitized hosts. Laboratory strains may be maintained in hosts and, in the case of some species, *in vitro* on cell cultures or embryos.

Experimental Culture in Hosts (*in vivo*)

Many apicomplexans may be maintained experimentally if their hosts can be bred in the lab. Infestation of a new host is generally accomplished orally. But in some cases infestation may occur by direct introduction of infectious stages into the body either by syringe inoculation or by an arthropod vector.

Gregarines. Some gregarine species may be bred and obtained in important quantities by experimental infection of the host, for example: *Gregarina blaberal,* parasitic on the cockroach *Blaberus cranifer*; *Gregarina rhyparabiae,* parasitic on the cockroach *Leucophaea maderae*; and *Gregarina garnhami,* parasitic on the locust *Locusta migratoria*. This breeding involves the following operations:

1) Production of specific pathogen-free hosts. The young insects are hatched from isolated eggs (or oothecae) in uncontaminated cages. Locusts are fed with young maize sprouts and cockroaches are fed with bran.

2) Collection of sporocysts: Gregarine cysts are normally collected from the host feces, cleaned and dried, and stored in a humid chamber until they sporulate.

3) Infection of young larval insects: Sporocysts from *in vitro* sporulation of cysts are deposited on the food and are eaten by larvae.

Coccidia. If infective forms are available, many coccidians may be kept by experimental infection of hosts derived from laboratory colonies. Culture of species with particular human medical or veterinary interest has been well studied. Various other species have been kept in cultures to resolve certain biological problems.

Grell (1953) succeeded in culturing the coccidian *Eucoccidium dinophili,* parasitic on the archiannelid worm *Dinophilus*. Hosts are bred in sea water and fed with a marine species of *Chlamydomonas* cultivated on Foyn medium. Because of the detritus-feeding character of the worms, the *Chlamydomonas* are killed by heat before being fed to the worms. Naturally infected worms are used to contaminate specific pathogen-free worms. Healthy worms are easily differentiated from infected ones by simple binocular microscope observation; the white opaque parasites may be clearly distinguished through the worms' transparent bodies. The experimental infection is carried out by mixing parasite sporocysts in the food.

Parasite development may be followed easily by simple microscopic observations. Such experimental infections allowed Grell to prove the absence of schizogony in this species and to make interesting observations on sex determination.

Most species of *Eimeria* may be easily kept in their hosts: for instance *E. necatrix* in chicken, *E. bovis* in calf, *E. nieschulzi* in rat. The following manipulations are required:

Oocysts must be set apart from feces of parasitized animals. These oocysts are generally unsporulated and they require a high level of oxygen in the medium to sporulate and form infectious sporozoites. Oocyst purification is carried out by floating, mixing, screening, and centrifugation. These oocysts may remain infectious for long periods if they are stored at 4°C.

Hosts are force-fed with a sufficient number of oocysts: e.g., 5×10^4 oocysts to 2-month-old chickens, 2×10^6 oocysts to 2-month-old calves and 5×10^5 oocysts to 200–300 gram rats.

Experimental cultures in natural hosts of heteroxenous apicomplexans may be carried out in the same way: setting up and concentrating infectious forms, and force-feeding the appropriate host. This process can be used with *Aggregata*: cuttlefish are force-fed infected crabs and the crabs are fed with infected cuttlefish organs. This is also possible with hosts of *Sarcocystis* and *Toxoplasma*.

Toxoplasma and *Besnoitia* may be kept indefinitely and easily in the growth phase on laboratory mice. Experimental transmission is accomplished as follows: A sample of peritoneal liquid is removed with a syringe from an infected mouse. An intraperitoneal injection of this liquid is then given to a specific pathogen-free mouse. The development is very fast and the new infection reaches a high level in a few days.

Hematozoa. Techniques for keeping heteroxenous coccidia in the laboratory are also available for hematozoans, but it is

necessary to be able to breed the vertebrate hosts and arthropod vectors in the laboratory. Various technical expedients may be useful for this breeding: splenectomy considerably increases parasitic development in mammalian hosts by reducing their resistance; hosts which are easily bred in the laboratory may be substituted for normal hosts; vector fasting (mosquitoes, for example) before a blood meal facilitates biting and parasite transmission; successive inoculations of parasites may increase the virulence of infection.

Various strains may be maintained almost indefinitely in their vertebrate host. Thus *Plasmodium chabaudi* may be kept in white mice by successive inoculation every 5 or 6 days: blood (3 drops) is taken from the tail of a mouse with a 2-day-old infection (60% parasitemia rate). This blood is mixed with 1.5 ml MEM (DIFCO) medium and 0.5 ml of the mixture is transferred intraperitoneally to another mouse ($5x10^5$ parasites are inoculated). When 60% of the red cells are infected, the strain may be transferred to other mice. Various *Plasmodium* and even some piroplasms may be kept in this way.

Cryopreservation may also be useful. Blood is mixed with 10% DMSO (10 blood volume for 1 medium volume). The mixture is divided into small 1 ml capsules and progressively frozen and stored at -196°C in liquid nitrogen. Later the mixture may be gradually warmed and inoculated into a new host.

Culture *in vitro*

It is not possible to cultivate apicomplexans on strictly artificial medium; a cellular base is required by the parasite. Nevertheless, test cultures on liquid medium have been accomplished with gregarines and coccidians that normally develop extracellularly in the coelomic fluid: the gregarine *Diplauxis hatti* (a coelomic parasite of *Perinereis cultrifera*) and the coccidian *Coelotropha durchoni* (a coelomic parasite of *Nereis diversicolor*). These two species only survive for a period; no development occurs.

Three cellular bases may be used for parasite cultures: bird embryos, organ cultures, and cell cultures. The first trials of coccidian culture on bird embryos and cell cultures were done in 1965. Organ culture is a recent technique and it seems that, so far, the parasite only survives, failing to develop.

Parasite cultures on chicken embryos have been carried out with various species of *Eimeria, Toxoplasma,* and *Besnoitia.* Eggs are infected by zoite inoculations (sporozoites, merozoites, or endozoites) in the allantoic cavity. Reproduction easily follows, and with *Eimeria* a portion of the life cycle may develop, depending on the species. The best result seems to be obtained with *E. tenella* (a species which normally develops in the chicken) where oocysts are produced after sporozoite or merozoite inoculations.

Probably the most successful and widely used technique involves cell cultures, which allow growth of many coccidian species, especially *Eimeria, Besnoitia, Toxoplasma, Sarcocystis, Isospora,* and more recently some hematozoans.

Cell cultures can be primary cultures from organs taken from an animal or an embryo (for instance kidney cells or fibroblasts) or cultures from a continuous cell line (the human tissue culture cells Hela or KB, for example). Classical techniques are used and a monocellular layer is obtained on the substrate. Infection with the parasite is carried out, under aseptic conditions, by inoculating with infective stages; Hela cell cultures (usually in MEM medium) are supplemented with 10% calf serum.

Many human and domestic animal pathogenic sporozoans may be cultured to study host-parasite relationships, immunity, drug action, or various aspects of parasite metabolism. *Toxoplasma gondii* and *Besnoitia* may grow indefinitely, reproduction occurring by an endogenous process. With other species capable of limited reproduction by mitosis (such as *Eimeria*), development in cell culture produces the first generation of merozoites, and sometimes a second generation of merozoites; with two species, complete development up to the oocyst has been achieved. *Sarcocystis* gamogony was shown *in vitro* by Fayer (1972) on a cell culture of bradyzoites (zoites in latent phase) from brain cysts before the complete life cycle was discovered.

In the piroplasms *Theileria parva* and *Theileria annulata,* macroschizozoites (zoites produced by large schizonts) have been cultured on lymphoblastoid cells transformed (capable of continuous growth) by the parasite from a continuous cell line. Lymphoid cells may be infected with sporozoites from ticks, and microschizozoites (zoites produced by small schizonts) have been obtained in culture (microschizozoites give rise to forms infecting erythrocytes). For *Babesia ovis,* the intraerythrocytic stages continuously grow in bovine red blood cells; the best results are obtained with a low level of oxygen in the culture.

A technique for *Plasmodium falciparum* cultures has been perfected by Trager and Jensen (1976). This technique uses an aqueous medium (called RPMI) with glucose, amino acids, and vitamins that contains 10% human or calf serum. Normal parasite-free and infected erythrocytes are introduced into the medium. Culture takes place under a high CO_2, low O_2 atmosphere; the medium is renewed every 24 hours. Under these conditions, both merogony and gamogony occur.

These new culture techniques are extremely effective in obtaining an adequate supply of a particular parasite stage for cytophysiological, biochemical, and physiological analysis. The principal results obtained with these techniques have been reviewed by Doran (1973) and more recently by Trager (1982).

EVOLUTIONARY HISTORY

Despite the lack of fossil remains, the probable evolution of the Apicomplexa may be deduced from that of the hosts. Indeed, in some cases the concomitant evolution of hosts and parasites has resulted in such specificity that one can identify the host species from its parasite (Théodoridès, 1984). Therefore, the gregarines, which are exclusively parasitic on invertebrates, surely evolved earlier than the other apicomplexan classes, which parasitize vertebrates.

The most informative groups with regard to the possible life cycle and cytological organization of apicomplexan ancestors are the Blastogregarinida and Archigregarinida, parasites of

marine annelids (Polychaeta). The sporozoites of the blastogregarine species *Siedleckia caulleryi* attach to the gut wall and develop into extracellular trophonts. The trophonts transform directly into gamonts without the preliminary pairing and encystment reported in other gregarines. Male gametes detach from their gamont and swim in the lumen of the gut toward the female gamont. The female gametes detach from the latter after fertilization. The resulting zygote secretes a thin envelope and gives rise to several sporozoites. Although the ultrastructure of the Blastogregarinida is not known, the description given by Chatton and Villeneuve (Grassé, 1953) suggests that the trophonts are similar to those of the Archigregarinida. One may indeed suppose that the apical complex persists in the trophonts and gamonts as is the case in the archigregarine *Selenidium hollandei* (Schrével, 1968) (Fig. 20).

The development of the blastogregarines suggests that the apicomplexan ancestor was a protoctist similar in form to a zoite (Figs. 3, 5). The original site of infection was probably the gut of archaic marine annelids. The parasites were probably attached to the gut wall and developed extracellularly. Fertilization followed by merogony is considered to be the primitive process of reproduction. Indeed, merogony has not been observed in the Archigregarinida in spite of earlier suppositions. Furthermore, the comparative development of Apicomplexa tends to suggest that the occurrence of merogony is associated with the intracellular or tissue location of the parasites. The penetration of host cells, development in tissues other than gut, merogony, and acquisition of a second host where merogony may be amplified, correspond therefore to further coevolution of parasites and hosts. Such adaptations are probably the consequences of the evolution of the host itself. Indeed, the coccidia of vertebrates are similar to the intracellular coccidia of invertebrates with respect to their life cycle and the ultrastructure of developmental stages.

The development of the hematozoans provides a superb example of coevolution of hosts and parasites. The differentiation of gametes and the beginning of sporogony occur in the gut of invertebrates (Diptera, Ixodidae), which were probably the hosts of ancestral hematozoans. The adaptation of hematozoans to hemotrophy has resulted in alterations of sporogony, the structure of the zoites, and the physiology of the parasites. Apicomplexan sporogony originally consisted of the dissemination in the external environment of zoites sheltered by a spore wall. In hematozoans, the direct inoculation of the zoites by the invertebrate into the vertebrate has resulted in a series of adaptations: a motile zygote (kinete) that penetrates the gut wall, accumulation of zoites in the salivary glands, and suppression of the spore wall. The small size of merozoites (about 1.5 μm in length) and their ability to ingest and digest the hemoglobin are adaptations to their development in erythrocytes.

To conclude, the Apicomplexa probably represent the most complex protoctistan parasites. The adhesion of the infecting stage to live cells, probably related to the origin of the apical complex, certainly has been important in the evolution of parasitism. The apical complex might correspond to the altered kinetid of a mastigote ancestor. Indeed, mastigotes such as the Bodonidae are capable of attaching themselves to a substratum. Furthermore, the occurrence of undulipodia in the male gametes of apicomplexans may be considered to be a mastigote feature.

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